Journal of Biomolecular Structure and Dynamics

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/tbsd20

# Pharmacological network study on the effect of 6-gingerol on cervical cancer using computerized databases

Fernando Martínez-Esquivias, Juan Manuel Guzmán-Flores, Ismael Fernando Chávez-Díaz, Laura Elena Iñiguez-Muñoz & Andrés Reyes-Chaparro

**To cite this article:** Fernando Martínez-Esquivias, Juan Manuel Guzmán-Flores, Ismael Fernando Chávez-Díaz, Laura Elena Iñiguez-Muñoz & Andrés Reyes-Chaparro (29 Sep 2023): Pharmacological network study on the effect of 6-gingerol on cervical cancer using computerized databases, Journal of Biomolecular Structure and Dynamics, DOI: 10.1080/07391102.2023.2264943

**To link to this article:** https://doi.org/10.1080/07391102.2023.2264943

JOURNAL OF BIOMOLECULAR STRUCTURE AND DYNAMICS
https://doi.org/10.1080/07391102.2023.2264943



# Pharmacological network study on the effect of 6-gingerol on cervical cancer using computerized databases

Fernando Martínez-Esquivias[a], Juan Manuel Guzmán-Flores[a], Ismael Fernando Chávez-Díaz[b], Laura Elena Iñiguez-Muñoz[c] and Andrés Reyes-Chaparro[d]

[a]Instituto de Investigación en Biociencias, Centro Universitario de Los Altos, Universidad de Guadalajara, Tepatitlán de Morelos, México; [b]Centro Nacional de Recursos Genéticos-INIFAP, Tepatitlán de Morelos, México; [c]Departamento de Ciencias de la Naturaleza, Centro Universitario del Sur, Universidad de Guadalajara, Ciudad Guzmán Municipio de Zapotlán el Grande, Jalisco, México; [d]Escuela Nacional de Ciencias Biologicas (ENCB) del Insituto Politécnico Nacional (IPN). Departamento de Morfología, Ciudad de México, México

Communicated by Ramaswamy H. Sarma

**ABSTRACT**

Cervical cancer (CC) is the most frequent cancer in the female population worldwide. Although there are treatments available, they are ineffective and cause adverse effects. 6-gingerol is an active component in ginger with anticancer activity. This research aims to discover the mechanism by which 6-gingerol act as an anticancer agent on CC through a pharmacological network using bioinformatics databases. From MalaCard, Swiss Target Prediction, Comparative Toxicogenomics Database, and Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform, we obtained the target genes for 6-gingerol and CC and matched them. We got 26 genes and analyzed them in ShinyGO-0.76.3 and DAVID-Bioinformatics Resources. Then, we generated a protein-protein interaction network in Cytoscape and obtained 12 hub genes. Hub genes were analyzed in Gene Expression Profiling Interactive Analysis and TISIDB. In addition, molecular docking studies were performed between target proteins with 6-gingerol using SwissDock database. Finally, molecular dynamics studies for three proteins with the lowest interaction energy were implemented using Gromacs software. According to gene ontology results, 6-gingerol is involved in processes of apoptosis, cell cycle, and protein kinase complexes, affecting mitochondria and pathways related to HPV infection. *CTNNB1* gene was negatively correlated with CD8+ infiltration but was not associated with a higher survival rate. Furthermore, the molecular docking study showed that 6-gingerol has a high binding to proteins, and the molecular dynamics showed a stable interaction of 6-gingerol to AKT1, CCNB1, and CTNNB1 proteins. Conclusion, our work helps to understand the anticancer activity of 6-gingerol in CC that should be studied experimentally.

**ARTICLE HISTORY**
Received 27 June 2023
Accepted 22 September 2023

**KEYWORDS**
6-gingerol; cervical cancer; pharmacological network; molecular docking; molecular dynamics

## Introduction

Cervical cancer (CC) continues to be today one of the main causes of female death worldwide, especially in less developed countries with deficiencies in the application of control and prevention measures to prevent (Zhang et al., 2020). The main risk factor for the development of CC is infection by oncogenic human papillomavirus (HPV). HPV causes lesions that, in most patients, usually disappear after a few months due to the intervention of the immune system. Still, in some patients, the lesions prevail and lead to the development of CC (Malik et al., 2023; Zhang et al., 2020).

According to the Global Cancer Observatory (GLOBOCAN), for 2020, it was reported that CC presented an incidence of 6.5% of newly reported cases and caused 7.7% of deaths in women worldwide (Sung et al., 2021). The most effective route to prevent the development of this disease is vaccination against HPV and early detection of precancerous lesions with the Papanicolaou test (McGraw & Ferrante, 2014). Once this disease is diagnosed, the treatments usually include surgery, radiotherapy, chemotherapy, or a combination. These treatments often cause life-threatening side effects in patients and often cause problems in other parts of the body (Wipperman et al., 2018). Currently, the natural components of plants have been proposed as effective anticancer agents without generating side effects (Gökalp, 2021; He et al., 2021).

Ginger (*Zingiber officinale*) is a plant known for its medicinal properties due to its antioxidant, anti-inflammatory, antimicrobial, and anticancer properties and its effect on preventing and controlling cardiovascular, neurodegenerative, respiratory, and respiratory diseases, obesity, and diabetes mellitus (Mao et al., 2019). Various components have been identified in ginger, the most important being gingerols, paradols, and shogoals. 6-gingerol is fresh ginger's most important functional component, with solid anticancer potential, but its mechanism of action is poorly understood (Zivarpour et al., 2021).

CONTACT Fernando Martínez-Esquivias fernando.mesquivias@academicos.udg.mx Instituto de Investigación en Biociencias, Centro Universitario de Los Altos, Universidad de Guadalajara, P.O. Box: 47600, Tepatitlán de Morelos, México

Supplemental data for this article can be accessed online at https://doi.org/10.1080/07391102.2023.2264943.

According to an *in vivo* study with mice and *in vitro* using HeLa cells by Rastogi *et al.*, the administration of 6-gingerol effectively inhibited cell proliferation by activating apoptosis mechanisms and suggested that this compound may help control and progression of CC. In addition, they demonstrated that combining 6-gingerol with cytoplastin increases oxidative stress and DNA damage and stimulates cancer cell death, supporting the therapeutic effect of cisplatin (Rastogi et al., 2015). Similarly, Kapoor et al., 2016 demonstrated that 6-gingerol generated cytotoxicity in the HeLa cell line and arrest of the G2 phase of the cell cycle. Furthermore, it was suggested that administration of 6-gingerol alone or combined with PI-3K inhibitor and cisplatin may provide better therapeutic effects for CC.

Currently, pharmacological network studies use computer databases to establish the protein-compound/disease-gene interaction to elucidate the mechanism of action of molecules with potentially medicinal effects. These strategies identify therapeutic targets matched with genes or proteins expressed in a specific disease, and protein-protein interaction networks of key molecules are generated to find hub genes. The results are validated by molecular docking and molecular dynamics to later give way to *in vitro* and *in vivo* experimental studies (Noor et al., 2022; Yuan et al., 2022). Therefore, this research aims to characterize the molecular mechanism of 6-gingerol in CC from a pharmacological network strategy using informatics tools. Figure 1 shows the general flow of the bioinformatics analyses performed.

## Methodology

### Data collection

From the PubChem website (https://pubchem.ncbi.nlm.nih.gov/compound/969516), the chemical structure and SMILES (simplified molecular input line entry specification) of 6-gingerol were obtained (Kim, 2021). Subsequently, we got the molecular targets for 6-gingerol for '*homo sapiens*' from the databases Swiss Target Prediction (http://www.swisstargetprediction.ch/) (Daina et al., 2019), Comparative Toxicogenomics (http://ctdbase.org/) (Davis et al., 2023), and Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform databases (TCMSP) (https://tcmsp-e.com/tcmsp.php) (Ru et al., 2014). Then, genes associated with CC were obtained from the MalaCards database, 'The human disease database' (https://www.malacards.org/). Finally, the intersection between the 6-gingerol targets and the CC target genes was obtained and visualized using the Venny 2.1.0 platform (https://bioinfogp.cnb.csic.es/tools/venny/).

### Gene ontology and pathway enrichment analyses

The 26 common genes between CC and 6-gingerol therapeutic targets were analyzed in the Shiny GO 0.76.3 database (http://bioinformatics.sdstate.edu/go/) to perform Gene Ontology enrichment analysis (GO), including analysis of Biological Processes (BP), Molecular Functions (MF) and Cellular Components (CCs) (Ge et al., 2020). In addition, the metabolic pathway enrichment was obtained from the DAVID database (https://david.ncifcrf.gov/tools.jsp) (Sherman et al., 2022). The cut-off criterion with an FDR $<0.05$ was taken into account.

### Protein-protein interaction network analysis

Subsequently, the list of proteins was analyzed in the Cytoscape v3.9.1 software. The STRING network file was downloaded to generate a protein-protein interaction network (PPI) and determine the degree of connectivity between proteins and known hub genes (Doncheva et al., 2019). We take into account a confidence limit (score) of 0.4 for the analysis.

### Survival curve and correlation analysis between hub genes and immune cell infiltration

We used the GEPIA2 database (http://gepia2.cancer-pku.cn/#index) to analyze the survival curves of the hub genes in Cervical and endocervical cancers (CESC) (Tang et al., 2019). A 95% confidence interval was used for the analysis. In addition, we analyzed hub genes for immune cell infiltration on CESC using the TISIDB database (http://cis.hku.hk/TISIDB/search.php) (Ru et al., 2019). Correlations equal to or greater than 0.300 were considered significant.

### Molecular docking

Before we performed the molecular docking between 6-gingerol and the target proteins (Table 2), the PDB files of the proteins were downloaded from the Research Collaboratory for Structural Bioinformatics (https://www.rcsb.org/) and AlphaFold Protein Structure Database (https://alphafold.ebi.ac.uk/) (Rose et al., 2021; Varadi et al., 2022). From the PubChem database (https://pubchem.ncbi.nlm.nih.gov) (Kim, 2021), the SDF file of the 6-gingerol structure was converted to the mol2 format with the OpenBabel software. Finally, the molecular docking was executed in the Swiss Institute of Bioinformatics database (http://www.swissdock.ch/) (The SIB Swiss Institute of Bioinformatics' resources: Focus on curated databases 2016), the result was visualized in the UCSF Chimera software and BIOVIA Discovery Studio (Huang et al., 2014; Sa et al., 2022).

### Molecular dynamics

Molecular dynamics tests were performed to evaluate the stability of 3 ligand-receptor complexes that resulted from the molecular docking tests and presented the lowest coupling energy. The charm-gui platform was used to prepare the different inputs, and the Gromacs 2021.1 software for molecular dynamics (Abraham et al., 2015; Jo et al., 2008; Lindahl et al., 2021). Each protein was preprocessed using the PDB reading tool (Jo et al., 2014). On the other hand, the docking ligand was changed to the mol2 format using OpenBabel (O'Boyle et al., 2011). The .mol2 file of the ligand was loaded into the Ligand Reader & Modeler tool to

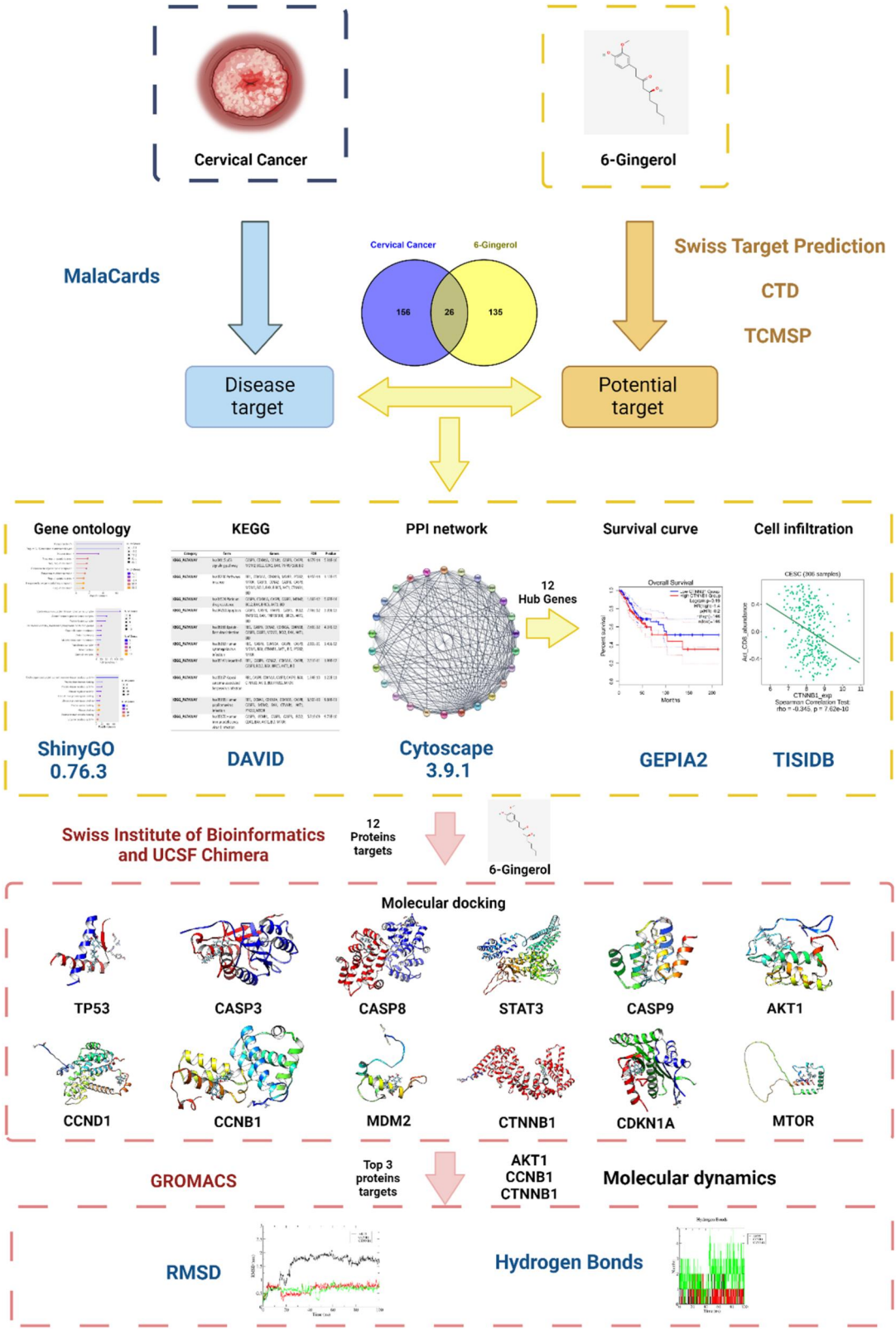


Figure 1. General flow of bioinformatics analysis.

generate the topology and parameter files (Kim et al., 2017). The ligand-receptor complexes were integrated into a single .pdb file that will be used in the 'Solution Builder' tool to create the system used as input for Gromacs (Lee et al., 2016). The water box was cubic, conforming to the size of the protein, and had an edge distance of 10 Å. Each system was neutralized using KCl ions placed by the Monte-Carlo method at a concentration of 0.15 M. Each system underwent 5000 steeper decay energy minimization steps to remove the steric overlap. Subsequently, all systems were subjected to a NVT (constant number of particles, volume, and temperature) equilibrium phase for 125,000 steps, using the V-rescale

temperature coupling method, with constant coupling of 1 ps at 303.15 K (Bussi et al., 2007). Subsequently, molecular dynamics was performed for 100 ns using the CHARMM36m force field (Vanommeslaeghe et al., 2010). Gromacs utilities were used to evaluate the root mean square deviation (RMSD) of the complexes and hydrogen bonding. Data was plotted using the GRACE program.

## Results

### Target analysis of 6-Gingerol and CC

Figure 2(A) shows the structure of 6-gingerol obtained from PubChem. The 6-gingerol targets identified were 161 genes, while genes related to CC were 182. By matching the CC genes to the 6-gingerol targets (Figure 2(B)), 26 genes were selected as potential targets for the therapeutic effect of 6-gingerol on CC.

### Analysis of gene ontology (GO) and metabolic pathways for overlapping targets

GO enrichment analysis of the 26 target genes showed that the important biological processes (BP) are related to the regulatory mechanisms of the cell cycle and apoptosis (Figure 3(A)). On the other hand, the cellular components (CCs) were related to mitochondria, protein kinase complex, outer organelle membrane, and transference complexes (Figure 3(B)). Molecular functions (MF) regulated protein kinase activity and ubiquitination processes (Figure 3(C)). Regarding the analysis of metabolic routes, a relationship was shown between the processes of apoptosis and viral infections mainly (Table 1).

### PPI network and key targets prediction

The main network obtained in STRING from Cytoscape presented 241 interactions between proteins (Figure 4), the nodes represent the target proteins, and the edges represent the interaction between the proteins. In addition, the proteins that presented more than 20 interactions were considered hub genes (Table 2).

### Correlation analysis between hub genes and immune cell infiltration and survival curve

The results of the correlation analysis between hub genes and the infiltration of cells of the immune system indicated only a negative correlation between the expression of *CTNNB1* and the infiltration of CD8+ lymphocytes on CESC (Supplementary Table 1). On the other hand, the survival analysis for *CTNNB1* was not associated with the probability of survival (Figure 5). Similarly, the rest of the hub genes were unrelated to the likelihood of survival (data not revealed).

### Molecular docking between 6-gingerol and target proteins

From the target proteins obtained from the PPI network (Table 2), we performed a molecular docking simulation with 6-gingerol. Figure 6 illustrates the results for the 12 target proteins. $\Delta G < 0$ suggests the possibility of spontaneous binding of 6-gingerol with proteins. The results showed that the $\Delta G$ of the 12 proteins was less than 0; this means that all the proteins spontaneously bind 6-gingerol (Figure 6).

### Molecular dynamics of top 3 protein targets

Considering that the environment of the solvent and the flexibility of the protein are not taken into account for the analysis of molecular coupling. We verified the veracity of the coupling results for the three target proteins that obtained the lowest coupling energy. The proteins selected for the 100 ns molecular dynamics were AKT1, CCNB1, and CTNNB1. In Figure 7(A), the RMSD results are shown; the CCNB1 and CTNNB1 complexes show stability at 1 nm, and the ligand remains in the binding site with a stable

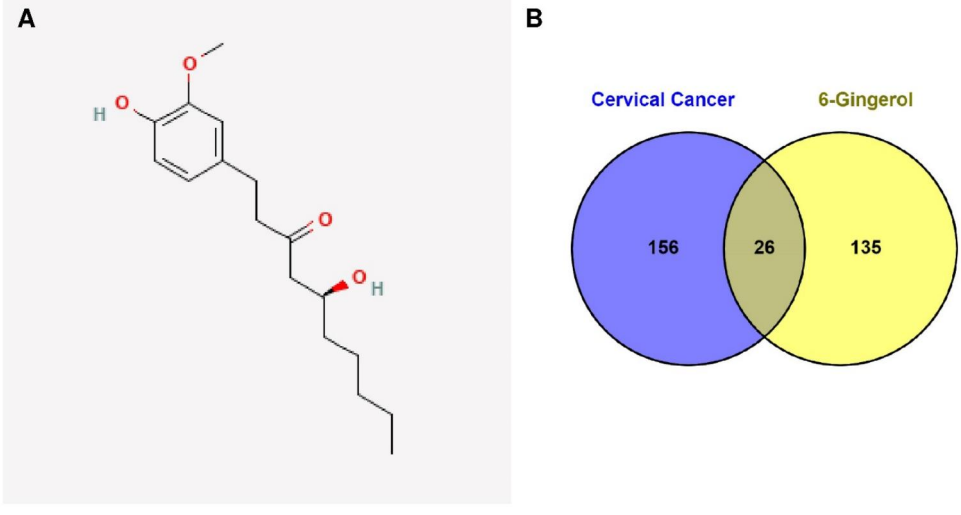


Figure 2. 6-Gingerol and CC. (A) Chemical structure of 6-Gingerol in 2D; (B) Venn diagram of the macheted genes between CC and 6-Gingerol targets.

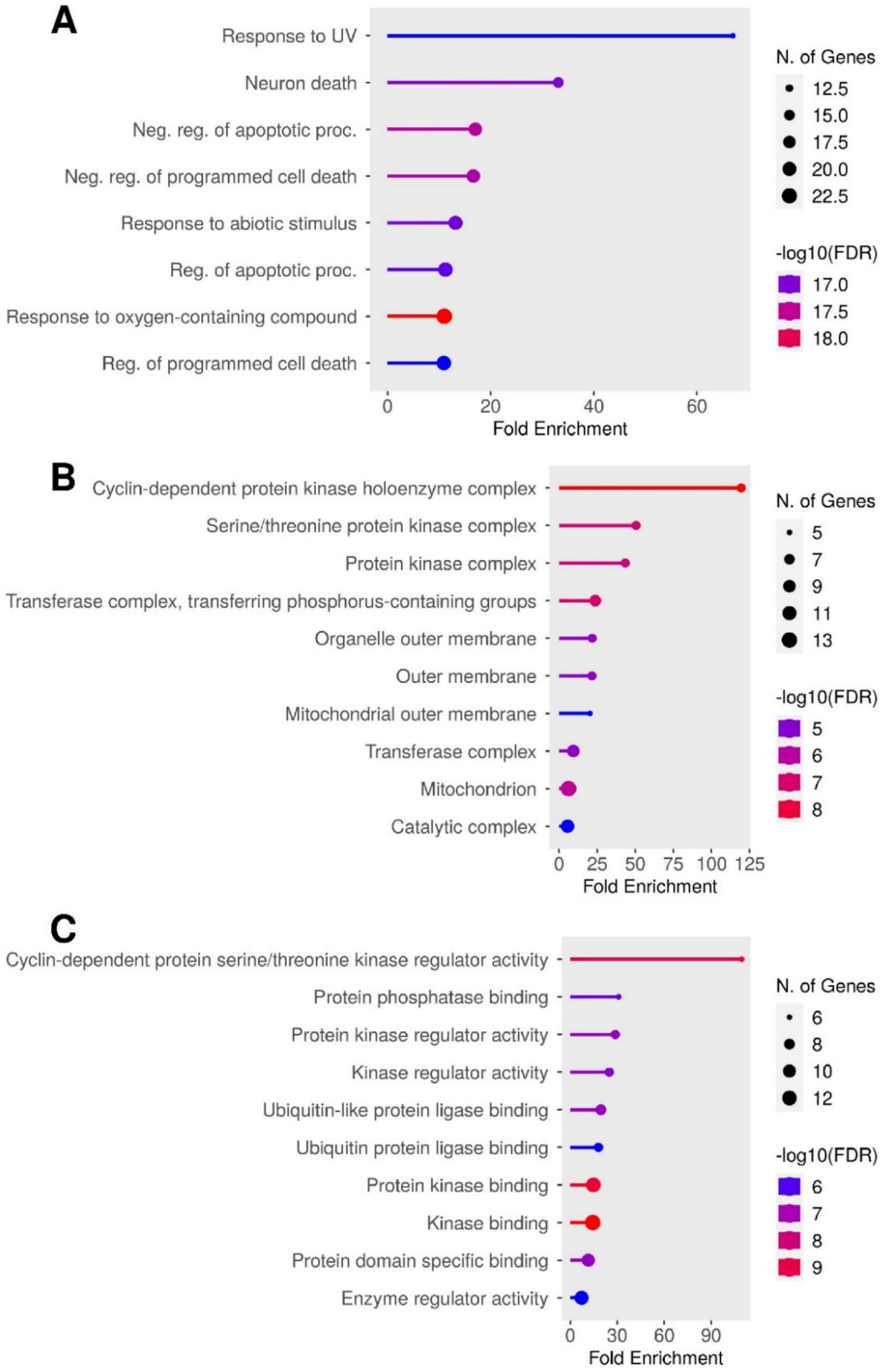


Figure 3. GO enrichment analysis for targets gene (top 10). (A) Biological Process. (B) Component Cellular (C) Molecular Functions.

interaction. On the other hand, the complex with the AKT1 protein shows a movement at 25 ns that remains until the end of the molecular dynamics; this indicates that after the ligand combines with the protein, the conformation of the protein will not change significantly. On the other hand, Figure 7(B) shows the results of hydrogen bonding for ligand-protein interactions. The interactions vary during molecular dynamics, being CTNNB1, the complex that achieves the highest number of interactions that give high stability to the molecule.

Table 1. Top 10 of pathway enrichment analysis of key targets.

| Term | Genes | FDR | p-value |
|---|---|---|---|
| hsa04115:p53 signaling pathway | *CASP9, CDKN1A, CCNB1, CASP8, CASP3, MDM2, BCL2, CDK1, BAX, TNFRSF10B, BID* | 4.67E-14 | 5.31E-16 |
| hsa05200:Pathways in cancer | *RB1, CDKN1A, CDKN1B, MMP2, PTGS2, MTOR, CASP9, CCNA2, CASP8, CASP3, MDM2, BCL2, BAX, BIRC5, AKT1, CTNNB1, BID* | 4.95E-14 | 1.12E-15 |
| hsa01524:Platinum drug resistance | *CASP9, CDKN1A, CASP8, CASP3, MDM2, BCL2, BAX, BIRC5, AKT1, BID* | 1.66E-12 | 5.67E-14 |
| hsa04210:Apoptosis | *CASP9, CASP8, PARP1, CASP3, BCL2, TNFSF10, BAX, TNFRSF10B, BIRC5, AKT1, BID* | 7.46E-12 | 3.39E-13 |
| hsa05169:Epstein-Barr virus infection | *RB1, CASP9, CCNA2, CDKN1A, CDKN1B, CASP8, CASP3, MDM2, BCL2, BAX, AKT1, BID* | 7.69E-12 | 4.37E-13 |
| hsa05163:Human cytomegalovirus infection | *RB1, CASP9, CDKN1A, CASP8, CASP3, MDM2, BAX, CTNNB1, AKT1, BID, PTGS2, MTOR* | 2.10E-11 | 1.43E-12 |
| hsa05161:Hepatitis B | *RB1, CASP9, CCNA2, CDKN1A, CASP8, CASP3, BCL2, BAX, BIRC5, AKT1, BID* | 2.51E-11 | 1.99E-12 |
| hsa05167:Kaposi sarcoma-associated herpesvirus infection | *RB1, CASP9, CDKN1A, CASP8, CASP3, BAX, CTNNB1, AKT1, BID, PTGS2, MTOR* | 1.34E-10 | 1.22E-11 |
| hsa05165:Human papillomavirus infection | *RB1, CCNA2, CDKN1A, CDKN1B, CASP8, CASP3, MDM2, BAX, CTNNB1, AKT1, PTGS2, MTOR* | 6.50E-10 | 9.60E-11 |
| hsa05170:Human immunodeficiency virus 1 infection | *CASP9, CCNB1, CASP8, CASP3, BCL2, CDK1, BAX, AKT1, BID, MTOR* | 5.71E-09 | 9.73E-10 |

Table 2. Hub genes for overlapping targets.

| Gene symbol | Protein | Function[a] | Degree |
|---|---|---|---|
| *TP53* | TP53-binding protein 1 | DNA double-strand break repair protein | 25 |
| *CASP3* | Capase 3 | Protein involved in the process of apoptosis | 25 |
| *CASP8* | Capase 8 | Protein involved in the process of apoptosis | 25 |
| *STAT3* | Signal transducer and activator of transcription 3 | Participates in the regulation of the cell cycle by inducing the expression of key genes for the progression from G1 to the S phase, such as CCND1 | 24 |
| *CASP9* | Caspase 9 | Protein involved in the process of apoptosis | 24 |
| *AKT1* | RAC-alfa serina/treonina-proteína quinasa | Enzyme regulating processes including metabolism, proliferation, cell survival, growth, and angiogenesis | 24 |
| *CCND1* | G1/S-specific cyclin-D1 | Phosphorylates and inhibits members of the retinoblastoma (RB) family of proteins, including RB1, and regulates the cell cycle during the G 1 /S transition | 23 |
| *CCNB1* | G2/mitotic-specific cyclin-B1 | Protein for the control of the cell cycle at the G2/M | 22 |
| *MDM2* | E3 ubiquitin-protein ligase Mdm2 | Inhibits cell cycle arrest and apoptosis mediated by p53/TP53 and p73/TP73 | 21 |
| *CTNNB1* | Catenin beta-1 | Protein regulates cell adhesion as a component of an E-cadherin: catenin adhesion complex. In addition, it participates in the negative regulation of centrosome cohesion | 21 |
| *CDKN1A* | BRCA2 and CDKN1A-interacting protein | Protein is required for the organization and anchoring activities of microtubules in interphase and is necessary for the organization and stabilization of the spindle pole in mitosis | 21 |
| *MTOR* | Serine/threonine-protein kinase mTOR | A central regulator of cell metabolism, growth, and survival in response to hormones, growth factors, nutrients, energy, and stress signals | 20 |

[a]Information is taken from the UniProt page (https://www.uniprot.org/).

## Discussion

Pharmacological network studies allow us to understand the actions of natural compounds and their interactions with multiple targets. It helps us integrate information related to the interaction between biological molecules, signaling pathways and disease networks to understand the mechanisms of action of natural compounds (Sabarathinam et al., 2023; Sabarathinam & Ganamurali, 2023).

Currently, CC continues to be a public health problem worldwide, and although there are available treatments, these are usually ineffective and can generate undesirable effects on patients. Many active components of natural origin with anticancer capacity could be used to treat this disease without adverse consequences for patients. In this investigation, we elucidate the mechanism of action of 6-gingerol on CC through a pharmacological network approach using bioinformatics databases. Our results confirm that 6-gingerol activates the mechanisms of cell apoptosis, we found 12 target genes in which 6-gingerol exerts its action, and we show that the proteins associated with these genes have a high probability of binding to 6-gingerol.

In this work, we found 26 differentially expressed genes in CC and 6-gingerol targets (Figure 2(B)). When we performed the analysis for GO, we found that the BP was mainly related to the mechanisms of cell apoptosis (Figure 3(A)). This finding is consistent with that reported in previous research (Kapoor et al., 2016; Rastogi et al., 2015). On the other hand, CCs and MF that could be affected by 6-gingerol administration include Cyclin-dependent and Serine-threonine protein kinase (Figure 3(B,C)). These enzyme complexes are involved in cell survival, cell cycle progression, and cell growth (Bhullar et al., 2018; Łukasik et al., 2021). According to Wang et al., (2014), 6-gingerol inhibits the biosynthesis of Cyclin-dependent protein kinases essential for the transition of the G1 and G2 phases in the cell division processes.

Similarly, Luo et al., 2019 showed that 6-gingerol combined with the cytoplastin inhibited cell migration and invasion, decreasing the expressions of cyclin D1, AKT, and AKT-phosphorylated proteins in gastric cancer cells. On the other hand, another CCs implicated is the mitochondria. According to Sp et al., (2021), 6-gingerol causes an increase in mitochondrial reactive oxygen species (ROS) that leads to apoptosis due to an elevation in *BAX*, *CYCS,* and *CASP9* and a decrease in the expression of *BCL-2* in breast cancer cell lines.

On the other hand, the metabolic pathways affected in CC by 6-gingerol include apoptosis processes, p53 signaling pathways, and HPV infection (Table 1). These findings agree with what was reported by Rastogi et al., 2015; they demonstrated that exposure to 6-gingerol inhibited cell proliferation and caused p53 activation, increased p21 levels, induced DNA damage and cell cycle arrest in the G2/M phase on HPV-positive CC cells. In addition, a previous computational

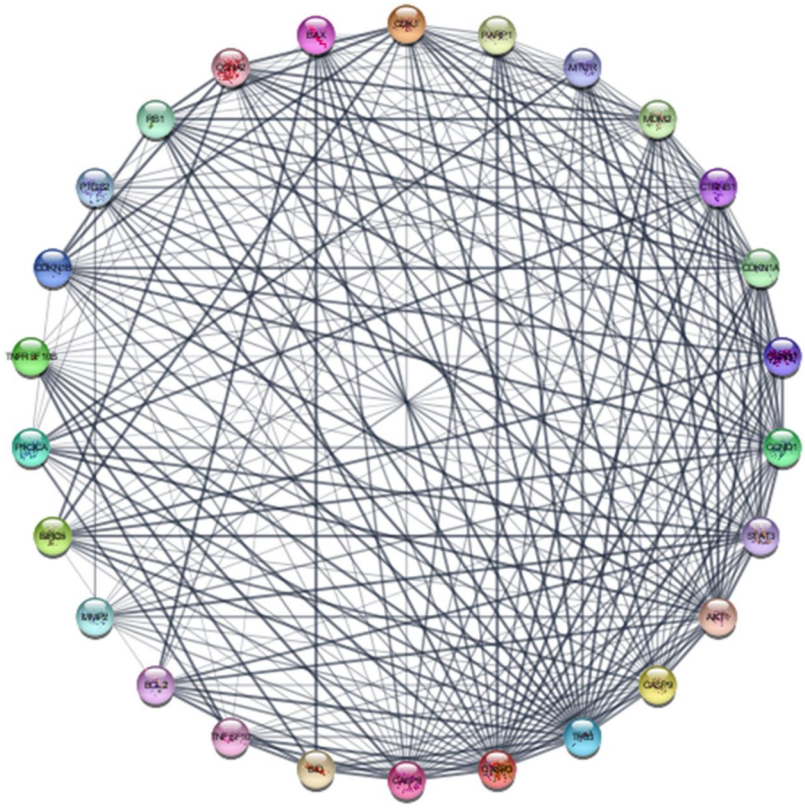

Figure 4. PPI Principal network constructed with the match targets from STRING.

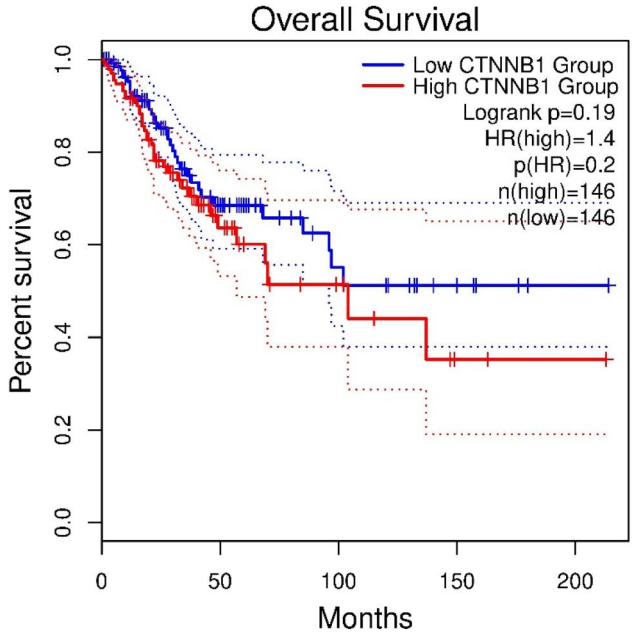


Figure 5. Survival curve of *CTNNB1* in CESC.

study demonstrated through simulations that 6-gingerol is an ideal drug candidate with antiviral activity due to inhibiting the E6 protein mechanism for HPV-16 (Kharisma et al., 2020).

From the PPI network, we found 12 hub genes (Table 2). We observed genes related to the processes of apoptosis *TP53*, *CASP3*, 8, and 9. As previously mentioned, 6-gingerol promotes cell death events by activating p53 (Rastogi et al., 2015). In addition, 6-gingerol has been associated with activating caspase 3, 8, and 9, which attests to the induction of apoptotic cell death (Radhakrishnan et al., 2014). We also observed genes related to cell cycle control, including *STAT3*, *CCND1*, *CCNB1*, *MDM2*, *CDKN1A*, *AKT1*, and *MTOR*. According to Lin et al., (2012), 6-gingerol causes increases in p53, p27, and p21 with a decrease in cyclin B1, cyclin A, and CDK1 leading to cell cycle arrest G2/M in the LoVo cell line. On the other hand, Xu et al., (2020) demonstrated that 6-gingerol causes G1 phase arrest through the AKT-GSK 3β-cyclin D1 pathway in cell lines 786-O, 769-P, and ACHN. Similarly, Sp et al., (2021) observed that the administration of 6-gingerol caused the downregulation of *CCND1* and *CDKN1A*. They demonstrated that 6-gingerol blocked the interaction of p53 with its negative regulator E3 ubiquitin-protein ligase MDM2 and suggested that 6-gingerol increases p53 expression by regulating EGFR/Src/STAT3 signaling in breast cancer cells. In addition, it was suggested that the anticancer activity of 6-gingerol is due to AMPK activation and inhibition of the AKT/mTOR signaling pathway in YD10B and Ca9-22 cell lines (Zhang et al., 2021).

Another of the target genes was *CTNNB1*, which codes for the β-catenin protein. It has recently been suggested that the dysregulation of this protein leads to the development of CC. It has been suggested as a potential prognostic marker and a drug target for cancer therapy (Wang et al., 2020). According to Lee et al., (2008), 6-gingerol deregulates the β-catenin pathway. This causes downregulation of cyclin D1 expression resulting in cell cycle arrest in colorectal cancer cells and contributing to its anticancer activity. Therefore, these 12 target genes are involved in anticancer activity and have been little studied in CC.

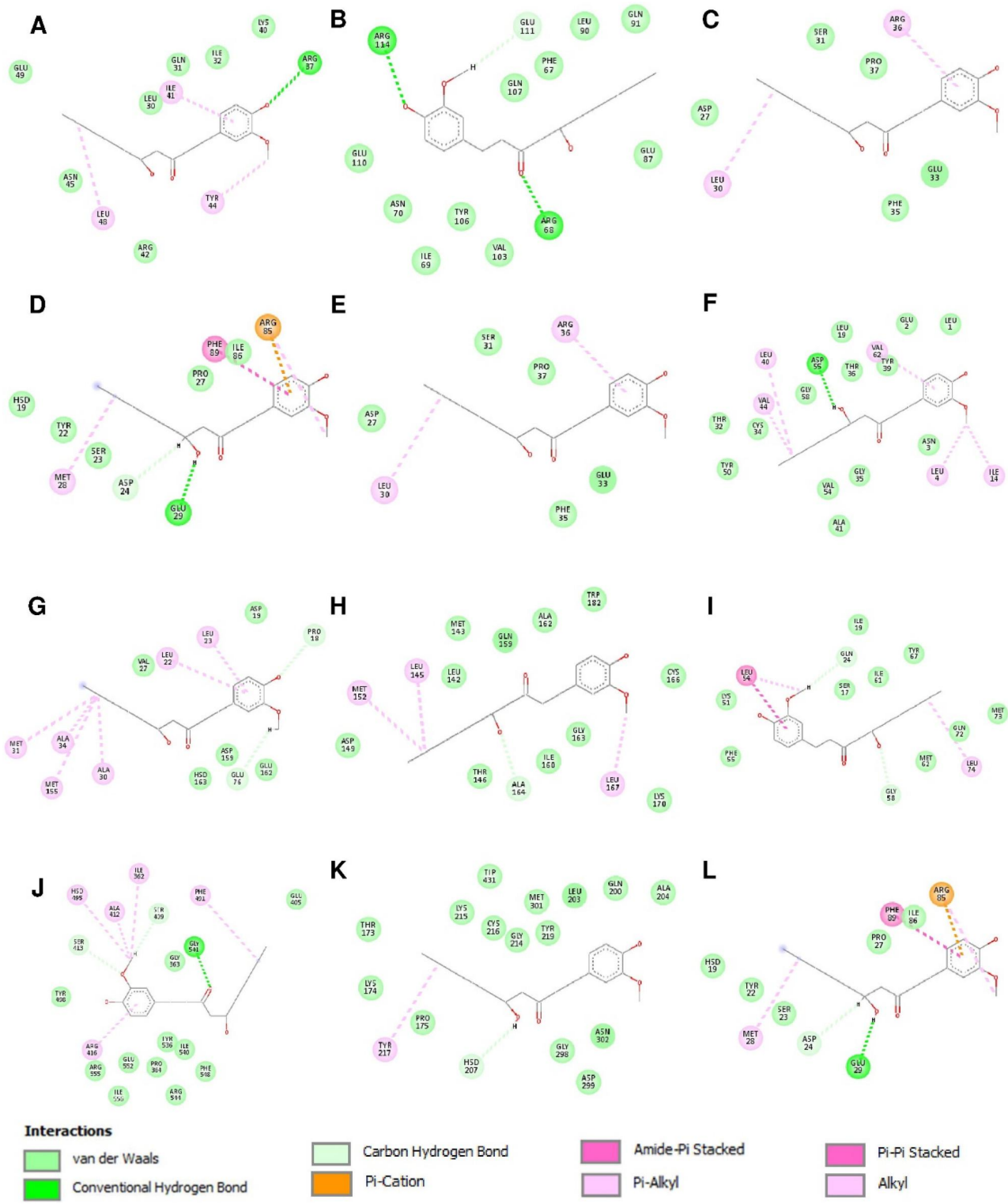


Figure 6. Interaction of 6-gingerol with proteinas targets. (A) TP53, ΔG=-7.18 kcal/mol; (B) CASP3, ΔG=-6.72 kcal/mol; (C) CASP8, ΔG=-6.97 kcal/mol; (D) STAT3, ΔG=-7.24 kcal/mol; (E) CASP9, ΔG=-6.75 kcal/mol; (F) AKT1, ΔG=-7.84 kcal/mol; (G) CCND1, ΔG=-7.15 kcal/mol; (H) CCNB1, ΔG=-7.33 kcal/mol; (I) MDM2, ΔG=-7.00 kcal/mol; (J) CTNNB1, ΔG=-8.11 kcal/mol; (K) CDKN1A, ΔG=-7.10 kcal/mol; (L) MTOR, ΔG=-7.24 kcal/mol.

In this investigation, we analyzed the target genes (Table 2) and their relationship with immune cell infiltration for CESC. The immune system cells are necessary to control tumor growth, and in the case of CC, the immune response is closely related to the HPV infection. Under this environment, the different cells of the immune system change during cancer development (Guo & Hua, 2020). We found a negative correlation between the infiltration of CD8+ lymphocytes and the *CTNNB1* gene. We previously mentioned that 6-gingerol was associated with β-catenin inhibition (Lee et al., 2008). Therefore, inhibiting β-catenin would increase the infiltration of CD8+ lymphocytes that can lyse HPV-infected cancer cells in patients with CC (Maskey et al., 2019; Santin et al., 1999). Furthermore, regarding the survival

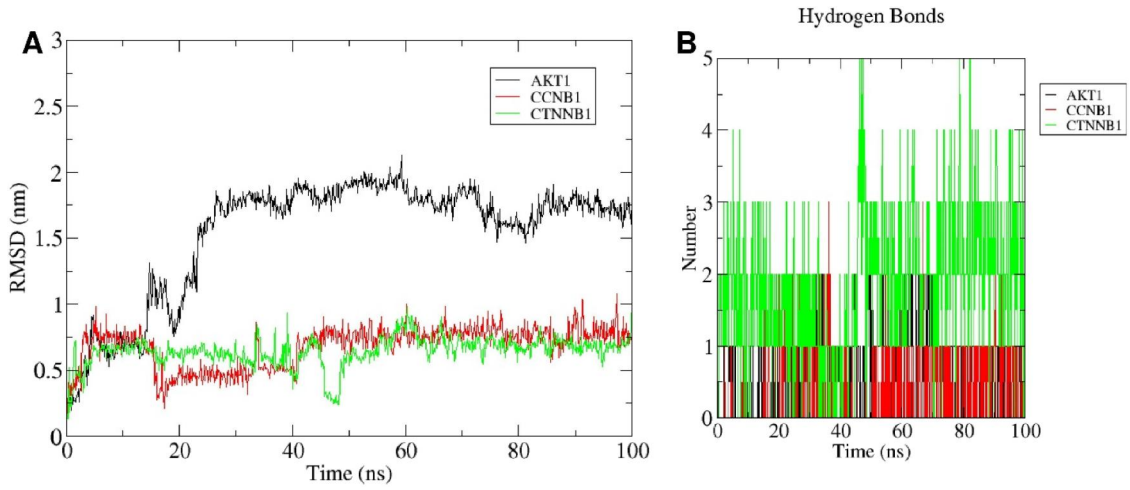


Figure 7. Molecular dynamics for AKT1, CCNB1, CTNNB1. (A) RMSD and (B) Hydrogen bonds.

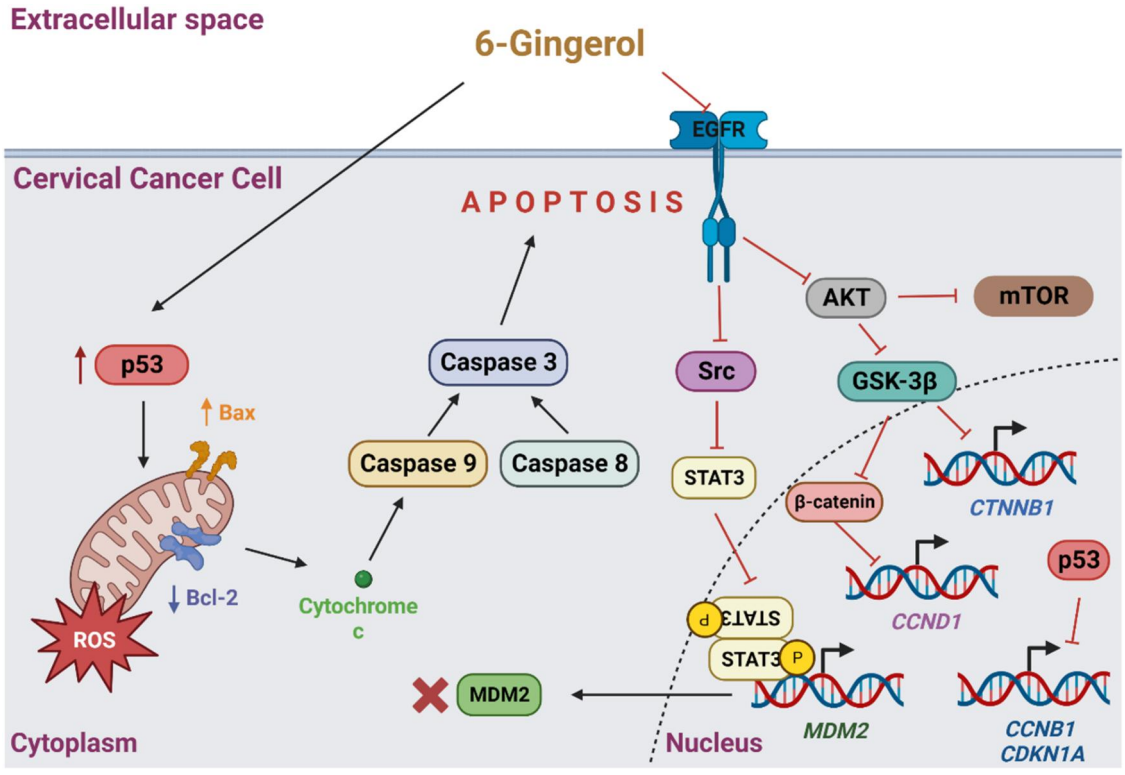


Figure 8. Anticancer effect of 6-gingerol. 6-gingerol increases the concentrations of p53, causing the mitochondria to release cytochrome c and increase the expression of the apoptotic protein bax and ROS. Cytochrome c activates caspase 8 and 9 to activate effector caspase 3 and promote apoptosis of cervical cancer cells. On the other hand, 6-gingerol plays an important role in cell cycle arrest. 6-gingerol causes an inhibition of the EGFR receptor signaling Cascade that affects the expression of genes related to the cell cycle.

analysis, we did not observe a significant effect between a low expression of CTNNB1 related to a higher survival rate (Figure 5). However, since 6-gingerol is related to the degradation of β-catenin, the administration could potentiate the effect and would mean an increase in the survival rate that should be confirmed experimentally.

On the other hand, the molecular docking results indicated that 6-gingerol showed high binding activity to 12 target proteins (Figure 6). According to the literature, the lower coupling energy indicates a stronger affinity of the coupled complex (Zhang et al., 2022). Therefore, these proteins are drug targets for 6-gingerol. As previously stated, 6-gingerol acts on these targets, but they have been little studied in CC.

Subsequently, we verified the molecular docking results of the AKT1, CCNB1, and CTNNB1 proteins through molecular dynamics simulation. The results obtained for RMSD and hydrogen bonding (Figure 7(A,B)) indicated the stability of 6-gingerol with AKT1, CCNB1, and CTNNB1. Therefore, this work encourages future research to corroborate the information obtained and complement it with exhaustive studies on pharmacokinetics in *in vivo* models. Although there are reports that have improved the bioavailability of 6-gingerol and have had a significant impact on the pharmacokinetic

profiles, more research is required in this area, toxicity studies and determination of the effective dose for the treatment of CC before administration in humans (Arcusa et al., 2022; Zivarpour et al., 2021). Finally, based on the information obtained in this investigation, in Figure 8, we propose the mechanism of action of 6-gingerol on CC.

## Conclusion

This pharmacological network study demonstrated that the anticancer effect of 6-gingerol focuses on the induction of the cellular apoptosis mechanism, causes cell cycle arrest, and affects mitochondria. In addition, we show that the downregulation of the *CTNNB1* gene by 6-gingerol leads to the infiltration of CD8+ lymphocytes that can destroy cancer cells. According to the molecular docking study we found that 6-gingerol binds highly to proteins related to hub genes. AKT1, CCNB1 and CTNNB1 proteins were subjected to molecular dynamics studies. The results showed that the interaction between 6-gingerol and protein was highly stable. This information indicates that these proteins are therapeutic targets for CC and future investigations should take into account the findings obtained for the development of experimental investigations.

## Acknowledgements

For the Consejo Nacional de Ciencia y Tecnologia (CONACYT) for supporting young researchers.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

## ORCID

*Andrés Reyes-Chaparro* http://orcid.org/0000-0002-6677-0740

## Author contributions

Conceptualization: F M-E; Methodology and software: F M-E, JM G-F, IF C-D, and A R-C; formal analysis and investigation: F M-E, JM G-F, IF C-D, LE-I-M, and A R-C; writing—review and editing: F M-E; JM G-F, IF C-D, LE I-M, and A R-C. All authors have read and agreed to the published version of the manuscript.

## References

Abraham, M. J., Murtola, T., Schulz, R., Páll, S., Smith, J. C., Hess, B., & Lindah, E. (2015). Gromacs: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX*, *1-2*, 19–25. https://doi.org/10.1016/j.softx.2015.06.001

Arcusa, R., Villaño, D., Marhuenda, J., Cano, M., Cerdà, B., & Zafrilla, P. (2022). Potential Role of Ginger (Zingiber officinale Roscoe) in the Prevention of Neurodegenerative Diseases. *Frontiers in Nutrition*, *9*, 809621. https://doi.org/10.3389/fnut.2022.809621

Bhullar, K. S., Lagarón, N. O., McGowan, E. M., Parmar, I., Jha, A., Hubbard, B. P., & Rupasinghe, H. P. V. (2018). Kinase-targeted cancer therapies: Progress, challenges and future directions. *Molecular Cancer*, *17*(1), 48. https://doi.org/10.1186/s12943-018-0804-2

Bussi, G., Donadio, D., & Parrinello, M. (2007). Canonical sampling through velocity rescaling. *The Journal of Chemical Physics*, *126*(1), 014101. https://doi.org/10.1063/1.2408420

Daina, A., Michielin, O., & Zoete, V. (2019). SwissTargetPrediction: Updated data and new features for efficient prediction of protein targets of small molecules. *Nucleic Acids Research*, *47*(W1), W357–W364. https://doi.org/10.1093/nar/gkz382

Davis, A. P., Wiegers, T. C., Johnson, R. J., Sciaky, D., Wiegers, J., & Mattingly, C. J. (2023). Comparative Toxicogenomics Database (CTD): Update 2023. *Nucleic Acids Research*, *51*(D1), D1257–D1262. https://doi.org/10.1093/nar/gkac833

Doncheva, N. T., Morris, J. H., Gorodkin, J., & Jensen, L. J. (2019). Cytoscape StringApp: Network analysis and visualization of proteomics data. *Journal of Proteome Research*, *18*(2), 623–632. https://doi.org/10.1021/acs.jproteome.8b00702

Ge, S. X., Jung, D., & Yao, R. (2020). ShinyGO: A graphical gene-set enrichment tool for animals and plants. *Bioinformatics (Oxford, England)*, *36*(8), 2628–2629. https://doi.org/10.1093/bioinformatics/btz931

Gökalp, F. (2021). The effective natural compounds for inhibiting Cervical cancer. *Medical Oncology*, *38*(2), 12. https://doi.org/10.1007/s12032-021-01456-3

Guo, L., & Hua, K. (2020). Cervical Cancer: Emerging Immune Landscape and Treatment. *OncoTargets and Therapy*, *13*, 8037–8047. https://doi.org/10.2147/OTT.S264312

He, M., Xia, L., & Li, J. (2021). Potential Mechanisms of Plant-Derived Natural Products in the Treatment of Cervical Cancer. *Biomolecules*, *11*(10), 1539. https://doi.org/10.3390/biom11101539

Huang, C. C., Meng, E. C., Morris, J. H., Pettersen, E. F., & Ferrin, T. E. (2014). Enhancing UCSF Chimera through web services. *Nucleic Acids Research*, *42*(Web Server issue), W478–484. https://doi.org/10.1093/nar/gku377

Jo, S., Cheng, X., Islam, S. M., Huang, L., Rui, H., Zhu, A., Lee, H. S., Qi, Y., Han, W., Vanommeslaeghe, K., MacKerell, A. D., Roux, B., & Im, W. (2014). CHARMM-GUI PDB manipulator for advanced modeling and simulations of proteins containing nonstandard residues. *Advances in Protein Chemistry and Structural Biology*, *96*, 235–265. https://doi.org/10.1016/bs.apcsb.2014.06.002

Jo, S., Kim, T., Iyer, V. G., & Im, W. (2008). CHARMM-GUI: A web-based graphical user interface for CHARMM. *Journal of Computational Chemistry*, *29*(11), 1859–1865. https://doi.org/10.1002/jcc

Kapoor, V., Aggarwal, S., & Das, S. N. (2016). 6-gingerol mediates its anti tumor activities in human oral and cervical cancer cell lines through apoptosis and cell cycle arrest. *Phytotherapy Research : PTR*, *30*(4), 588–595. https://doi.org/10.1002/ptr.5561

Kharisma, V. D., Ansori, A. N. M., & Nugraha, A. P. (2020). Computational study of ginger (Zingiber Officinale) as E6 inhibitor in human papillomavirus type 16 (Hpv-16) infection. *Biochemical and Cellular Archives*, *20*, 3155–3159. https://doi.org/10.35124/bca.2020.20.S1.3155

Kim, S. (2021). Exploring Chemical Information in PubChem. *Current Protocols*, *1*(8), e217. https://doi.org/10.1002/cpz1.217

Kim, S., Lee, J., Jo, S., Brooks, C. L., Lee, H. S., & Im, W. (2017). CHARMM-GUI ligand reader and modeler for CHARMM force field generation of small molecules. *Journal of Computational Chemistry*, *38*(21), 1879–1886. https://doi.org/10.1002/jcc.24829

Lee, J., Cheng, X., Swails, J. M., Yeom, M. S., Eastman, P. K., Lemkul, J. A., Wei, S., Buckner, J., Jeong, J. C., Qi, Y., Jo, S., Pande, V. S., Case, D. A., Brooks, C. L., MacKerell, A. D., Klauda, J. B., & Im, W. (2016). CHARMM-GUI input generator for NAMD, GROMACS, AMBER, OpenMM, and CHARMM/OpenMM simulations using the CHARMM36 Additive Force Field. *Journal of Chemical Theory and Computation*, *12*(1), 405–413. https://doi.org/10.1021/acs.jctc.5b00935

Lee, S.-H., Cekanova, M., & Baek, S. J. (2008). Multiple mechanisms are involved in 6-gingerol-induced cell growth arrest and apoptosis in human colorectal cancer cells. *Molecular Carcinogenesis*, *47*(3), 197–208. https://doi.org/10.1002/mc.20374

Lin, C.-B., Lin, C.-C., & Tsay, G. J. (2012). 6-gingerol inhibits growth of colon cancer cell LoVo via Induction of G2/M arrest. *Evidence-Based Complementary and Alternative Medicine : eCAM*, *2012*, 326096. https://doi.org/10.1155/2012/326096

Lindahl, Abraham, Hess, Spoel, & van der. (2021). *GROMACS 2021 Source code*. https://doi.org/10.5281/ZENODO.4457591

Łukasik, P., Załuski, M., & Gutowska, I. (2021). Cyclin-Dependent Kinases (CDK) and Their Role in Diseases Development–Review. *International Journal of Molecular Sciences*, *22*(6), 2935. https://doi.org/10.3390/ijms22062935

Luo, Y., Zha, L., Luo, L., Chen, X., Zhang, Q., Gao, C., Zhuang, X., Yuan, S., & Qiao, T. (2019). [6]-Gingerol enhances the cisplatin sensitivity of gastric cancer cells through inhibition of proliferation and invasion via PI3K/AKT signaling pathway. *Phytotherapy Research : PTR*, *33*(5), 1353–1362. https://doi.org/10.1002/ptr.6325

Malik, S., Sah, R., Muhammad, K., & Waheed, Y. (2023). Tracking HPV infection, associated cancer development, and recent treatment efforts—a comprehensive review. *Vaccines*, *11*(1), 102. Article 1. https://doi.org/10.3390/vaccines11010102

Mao, Q.-Q., Xu, X.-Y., Cao, S.-Y., Gan, R.-Y., Corke, H., Beta, T., & Li, H.-B. (2019). Bioactive Compounds and Bioactivities of Ginger (Zingiber officinale Roscoe). *Foods (Basel, Switzerland)*, *8*(6), 185. https://doi.org/10.3390/foods8060185

Maskey, N., Thapa, N., Maharjan, M., Shrestha, G., Maharjan, N., Cai, H., & Liu, S. (2019). Infiltrating CD4 and CD8 lymphocytes in HPV infected uterine cervical milieu. *Cancer Management and Research*, *11*, 7647–7655. https://doi.org/10.2147/CMAR.S217264

McGraw, S. L., & Ferrante, J. M. (2014). Update on prevention and screening of cervical cancer. *World Journal of Clinical Oncology*, *5*(4), 744–752. https://doi.org/10.5306/wjco.v5.i4.744

Noor, F., Tahir Ul Qamar, M., Ashfaq, U. A., Albutti, A., Alwashmi, A. S. S., & Aljasir, M. A. (2022). Network Pharmacology Approach for Medicinal Plants: Review and Assessment. *Pharmaceuticals (Basel, Switzerland)*, *15*(5), 572. https://doi.org/10.3390/ph15050572

O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. (2011). Open Babel: An open chemical toolbox. *Journal of Cheminformatics*, *3*(1), 33. https://doi.org/10.1186/1758-2946-3-33

Radhakrishnan, E., Bava, S. V., Narayanan, S. S., Nath, L. R., Thulasidasan, A. K. T., Soniya, E. V., & Anto, R. J. (2014). [6]-gingerol induces caspase-dependent apoptosis and prevents pma-induced proliferation in colon cancer cells by inhibiting MAPK/AP-1 signaling. *PloS One*, *9*(8), e104401. https://doi.org/10.1371/journal.pone.0104401

Rastogi, N., Duggal, S., Singh, S. K., Porwal, K., Srivastava, V. K., Maurya, R., Bhatt, M. L. B., & Mishra, D. P. (2015). Proteasome inhibition mediates p53 reactivation and anti-cancer activity of 6-gingerol in cervical cancer cells. *Oncotarget*, *6*(41), 43310–43325. https://doi.org/10.18632/oncotarget.6383

Rose, Y., Duarte, J. M., Lowe, R., Segura, J., Bi, C., Bhikadiya, C., Chen, L., Rose, A. S., Bittrich, S., Burley, S. K., & Westbrook, J. D. (2021). RCSB protein data bank: Architectural advances towards integrated searching and efficient access to macromolecular structure data from the PDB archive. *Journal of Molecular Biology*, *433*(11), 166704. https://doi.org/10.1016/j.jmb.2020.11.003

Ru, B., Wong, C. N., Tong, Y., Zhong, J. Y., Zhong, S. S. W., Wu, W. C., Chu, K. C., Wong, C. Y., Lau, C. Y., Chen, I., Chan, N. W., & Zhang, J. (2019). TISIDB: An integrated repository portal for tumor-immune system interactions. *Bioinformatics (Oxford, England)*, *35*(20), 4200–4202. https://doi.org/10.1093/bioinformatics/btz210

Ru, J., Li, P., Wang, J., Zhou, W., Li, B., Huang, C., Li, P., Guo, Z., Tao, W., Yang, Y., Xu, X., Li, Y., Wang, Y., & Yang, L. (2014). TCMSP: A database of systems pharmacology for drug discovery from herbal medicines. *Journal of Cheminformatics*, *6*(1), 13. https://doi.org/10.1186/1758-2946-6-13

Sa, K., Y, W., As, L., Xq, F., & Zl, Y. (2022). Network pharmacology and molecular docking-based prediction of active compounds and mechanisms of action of Cnidii Fructus in treating atopic dermatitis. *BMC Complementary Medicine and Therapies*, *22*(1), 275. https://doi.org/10.1186/s12906-022-03734-7

Sabarathinam, S., & Ganamurali, N. (2023). Chalcones reloaded: An integration of network pharmacology and molecular docking for type 2 diabetes therapy. *Journal of Biomolecular Structure & Dynamics*, 1–13. https://doi.org/10.1080/07391102.2023.2252085

Sabarathinam, S., Rajappan Chandra, S. K., & Satheesh, S. (2023). Network pharmacology based pharmacokinetic assessment and evaluation of the therapeutic potential of catechin derivatives as a potential myostatin inhibitor: A special view on Sarcopenic Obesity. *Natural Product Research*, 1–5. https://doi.org/10.1080/14786419.2023.2191197

Santin, A. D., Hermonat, P. L., Ravaggi, A., Chiriva-Internati, M., Zhan, D., Pecorelli, S., Parham, G. P., & Cannon, M. J. (1999). Induction of human papillomavirus-specific CD4+ and CD8+ Lymphocytes by E7-pulsed autologous dendritic cells in patients with human papillomavirus type 16- and 18-positive cervical cancer. *Journal of Virology*, *73*(7), 5402–5410. https://doi.org/10.1128/JVI.73.7.5402-5410.1999

Sherman, B. T., Hao, M., Qiu, J., Jiao, X., Baseler, M. W., Lane, H. C., Imamichi, T., & Chang, W. (2022). DAVID: A web server for functional enrichment analysis and functional annotation of gene lists (2021 update). *Nucleic Acids Research*, *50*(W1), W216–W221. https://doi.org/10.1093/nar/gkac194

Sp, N., Kang, D. Y., Lee, J.-M., Bae, S. W., & Jang, K.-J. (2021). Potential Antitumor Effects of 6-Gingerol in p53-dependent mitochondrial apoptosis and inhibition of tumor sphere formation in breast cancer cells. *International Journal of Molecular Sciences*, *22*(9), 4660. https://doi.org/10.3390/ijms22094660

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, *71*(3), 209–249. https://doi.org/10.3322/caac.21660

Tang, Z., Kang, B., Li, C., Chen, T., & Zhang, Z. (2019). GEPIA2: An enhanced web server for large-scale expression profiling and interactive analysis. *Nucleic Acids Research*, *47*(W1), W556–W560. https://doi.org/10.1093/nar/gkz430

The SIB Swiss Institute of Bioinformatics' resources: Focus on curated databases. (2016). *Nucleic Acids Research*, *44*(Database issue), D27–D37. https://doi.org/10.1093/nar/gkv1310

Vanommeslaeghe, K., Hatcher, E., Acharya, C., Kundu, S., Zhong, S., Shim, J., Darian, E., Guvench, O., Lopes, P., Vorobyov, I., & Mackerell, A. D. (2010). CHARMM general force field: A force field for drug-like molecules compatible with the CHARMM all-atom additive biological force fields. *Journal of Computational Chemistry*, *31*(4), 671–690. https://doi.org/10.1002/jcc.21367

Varadi, M., Anyango, S., Deshpande, M., Nair, S., Natassia, C., Yordanova, G., Yuan, D., Stroe, O., Wood, G., Laydon, A., Žídek, A., Green, T., Tunyasuvunakool, K., Petersen, S., Jumper, J., Clancy, E., Green, R., Vora, A., Lutfi, M., … Velankar, S. (2022). AlphaFold Protein Structure Database: Massively expanding the structural coverage of protein-sequence space with high-accuracy models. *Nucleic Acids Research*, *50*(D1), D439–D444. https://doi.org/10.1093/nar/gkab1061

Wang, B., Li, X., Liu, L., & Wang, M. (2020). β-Catenin: Oncogenic role and therapeutic target in cervical cancer. *Biological Research*, *53*(1), 33. https://doi.org/10.1186/s40659-020-00301-7

Wang, S., Zhang, C., Yang, G., & Yang, Y. (2014). Biological properties of 6-gingerol: A brief review. *Natural Product Communications*, *9*(7), 1934578X1400900. https://doi.org/10.1177/1934578X1400900736

Wipperman, J., Neil, T., & Williams, T. (2018). Cervical Cancer: Evaluation and Management. *American Family Physician*, *97*(7), 449–454.

Xu, S., Zhang, H., Liu, T., Yang, W., Lv, W., He, D., Guo, P., & Li, L. (2020). 6-Gingerol induces cell-cycle G1-phase arrest through AKT–GSK 3β–cyclin D1 pathway in renal-cell carcinoma. *Cancer Chemotherapy and Pharmacology*, *85*(2), 379–390. https://doi.org/10.1007/s00280-019-03999-9

Yuan, Z., Pan, Y., Leng, T., Chu, Y., Zhang, H., Ma, J., & Ma, X. (2022). Progress and Prospects of Research Ideas and Methods in the Network Pharmacology of Traditional Chinese Medicine. *Journal of Pharmacy & Pharmaceutical Sciences : a Publication of the Canadian Society for Pharmaceutical Sciences, Societe Canadienne Des Sciences Pharmaceutiques*, *25*, 218–226. https://doi.org/10.18433/jpps32911

Zhang, H., Kim, E., Yi, J., Hai, H., Kim, H., Park, S., Lim, S.-G., Kim, S.-Y., Jang, S., Kim, K., Kim, E.-K., Lee, Y., Ryoo, Z., & Kim, M. (2021). [6]-Gingerol Suppresses Oral Cancer Cell Growth by Inducing the Activation of AMPK and Suppressing the AKT/mTOR Signaling

Pathway. *In Vivo (Athens, Greece)*, *35*(6), 3193–3201. https://doi.org/10.21873/invivo.12614

Zhang, S., Xu, H., Zhang, L., & Qiao, Y. (2020). Cervical cancer: Epidemiology, risk factors and screening. *Chinese Journal of Cancer Research = Chung-Kuo Yen Cheng Yen Chiu*, *32*(6), 720–728. https://doi.org/10.21147/j.issn.1000-9604.2020.06.05

Zhang, X., Chen, M., Fan, P., Su, T., Liang, S., & Jiang, W. (2022). Prediction of the Mechanism of Sodium Butyrate against Radiation-Induced Lung Injury in Non-Small Cell Lung Cancer Based on Network Pharmacology and Molecular Dynamic Simulations. *Frontiers in Oncology*, *12*, 809772. https://doi.org/10.3389/fonc.2022.809772

Zivarpour, P., Nikkhah, E., Maleki Dana, P., Asemi, Z., & Hallajzadeh, J. (2021). Molecular and biological functions of gingerol as a natural effective therapeutic drug for cervical cancer. *Journal of Ovarian Research*, *14*(1), 43. https://doi.org/10.1186/s13048-021-00789-x