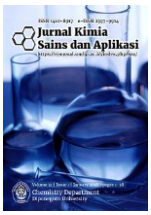
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Identification of CCNA2 as a Therapeutic Target in Cholangiocarcinoma and Evaluation of Indonesian *Jamu*-Derived Phytochemicals through Network Pharmacology and Molecular Docking

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Abstract

Cholangiocarcinoma (CCA) remains a highly aggressive malignancy with limited therapeutic options and poor prognosis, particularly in Southeast Asia, including Indonesia. The identification of novel molecular targets is essential to improve treatment strategies. This study aimed to identify key therapeutic targets in CCA and evaluate the potential of Indonesian *jamu*-derived phytochemicals as candidate inhibitors using an integrated computational approach. Differentially expressed genes (DEGs) associated with CCA were analyzed and intersected with disease-related datasets to construct a protein–protein interaction (PPI) network, from which hub genes were identified. Among these, CCNA2, a critical regulator of cell cycle progression, was selected as the primary target due to its central role in tumor proliferation and strong network connectivity. Subsequently, selected phytochemicals derived from Indonesian medicinal plants commonly used in *jamu*, including *Curcuma longa*, *Curcuma xanthorrhiza*, *Zingiber officinale*, and *Kaempferia galanga*, were screened for ethnobotanical relevance, their reported anticancer activities in modulating cell cycle progression, and drug-likeness using Lipinski's rule of five. Molecular docking analysis was performed to evaluate the binding affinity of these compounds against CCNA2. Several phytochemicals demonstrated favorable binding energies comparable to or exceeding that of the native ligand (-8.8 kcal/mol), indicating strong interaction potential within the active site of the protein. The binding interactions were stabilized through hydrogen bonding, hydrophobic interactions, and π- related interactions, suggesting a plausible mechanism for inhibitory activity. This study highlights CCNA2 as a promising therapeutic target in CCA and demonstrates the potential of Indonesian *jamu* phytochemicals as candidate inhibitors. These findings provide a scientific basis for further experimental validation and support the integration of traditional herbal medicine into modern drug discovery pipelines for cholangiocarcinoma treatment.

1. Introduction

Cholangiocarcinoma (CCA) represents a significant and increasingly recognized malignancy in Asia. The incidence of CCA reaches 0.3-6 per 100,000 people per year, with a mortality rate of 1–6 per 100,000 people per year [1]. The epidemiological patterns are influenced by regional risk factors such as chronic liver inflammation, hepatobiliary diseases, and environmental exposures. CCA contributes substantially to liver cancer–related mortality, with outcomes remaining poor due to late-stage diagnosis and limited treatment efficacy. Previous studies have highlighted that CCA is often asymptomatic in early stages, leading to delayed clinical detection and a 5-year survival rate below 9% [2, 3, 4]. In Southeast Asian

populations, including neighboring endemic regions, the burden is even higher due to additional risk factors such as liver fluke infection and chronic biliary inflammation [5]. These findings emphasize the urgent need for improved strategies in early detection and targeted therapy development, particularly in countries like Indonesia, where healthcare access and diagnostic capacity may vary.

Understanding the molecular mechanisms underlying CCA progression is essential for identifying novel therapeutic targets and improving treatment outcomes. Recent advances in transcriptomics and systems biology have revealed that CCA development is driven by complex dysregulation of signaling pathways, particularly those involved in cell cycle control, apoptosis, and proliferation [6]. Key regulators such as cyclins, cyclin-dependent kinases, and mitotic checkpoint proteins have been shown to play critical roles in tumor growth and progression [7]. Targeting these hub genes is considered a promising strategy because their inhibition can disrupt multiple downstream pathways simultaneously [6].

In parallel, the exploration of natural products as therapeutic agents has gained increasing attention, particularly in biodiverse countries like Indonesia. *Jamu*, a traditional herbal drink from Indonesia, was officially designated as an Intangible Cultural Heritage (ICH) by UNESCO on December 6, 2023 [8]. It has been widely used for generations to help prevent and treat a variety of diseases [9]. This herbal preparation typically consists of a combination of medicinal plants, including turmeric (*Curcuma longa*), curcuma (*Curcuma xanthorrhiza*), ginger (*Zingiber officinale*), aromatic ginger (*Kaempferia galanga*), bitter ginger (*Zingiber zerumbet*), and finger root (*Boesenbergia pandurata*). These medicinal plants are rich in bioactive phytochemicals. including curcumin, xanthorrhizol, gingerol, kaempferol, quercetin, boesenbergin, and others, which have demonstrated various pharmacological activities, including anticancer, anti-inflammatory, and antioxidant effects [10]. Importantly, recent studies have shown that phytochemicals derived from these plants can modulate key cancer-related pathways, including cell cycle regulation and apoptosis, making them promising candidates for targeted therapy [11, 12, 13, 14].

Notably, several of these compounds exhibit anticancer effects through modulation of key signaling pathways and cell cycle regulation. For instance, curcumin, a key curcuminoid in *C. longa*, inhibits the PI3K/Akt/mTOR kinase pathway by suppressing Akt phosphorylation, which directly leads to the downregulation of cyclin D1, cyclin B1, and CDK1 (CDC2), alongside the upregulation of the CDK inhibitor p21. This combined effect induces G1/S or G2/M phase cell cycle arrest. Simultaneously, curcumin shifts the cellular apoptosis balance by decreasing anti-apoptotic bcl-2 expression, enhancing bax accumulation, and activating executioner caspase-3 [15, 16]. Curcumin also enhances p53 expression, followed by increased p21 (WAF-1/CIP-1), leading to cell cycle arrest at the G0/G1 and/or G2/M phases [12]. Quercetin and kaempferol, the major flavonoids from *Z. zerumbet* and *K. galanga*, exert ATP-competitive inhibition against upstream regulatory kinases (including PI3K and PKC), resulting in the suppression of surviving and cyclin B1/CDK1 activity, which subsequently triggers mitochondrial-mediated apoptosis via caspase-9 and caspase-3 activation [17, 18]. In addition, Boesenbergin A, isolated from *B. pandurata*, has been reported to induce G2/M phase cell cycle arrest in human T4-lymphoblastoid cells [19].

Network-based approaches, including protein–protein interaction (PPI) analysis and hub gene identification, have further enabled the prioritization of central genes, which function as critical nodes in maintaining cancer cell survival. Integrating bioinformatics analyses with molecular docking provides a rational framework for identifying and validating potential therapeutic targets in CCA. By combining ethnopharmacological knowledge with modern computational approaches such as molecular docking and drug-likeness screening, *jamu*-derived phytochemicals can be systematically evaluated as potential inhibitors of key targets, offering a novel and culturally relevant strategy for CCA drug discovery.

2. Experimental

2.1. Cholangiocarcinoma Gene Mining

The cholangiocarcinoma genes were retrieved from GeneCards (https://www.genecards.org/). As this study focused on cholangiocarcinoma tumor signaling, proliferation, and resistance pathways, multiple keywords were used in a pathway-based search, including “Cholangiocarcinoma”, “Cholangiocarcinoma proliferation”, “Cholangiocarcinoma resistance”, and “Cholangiocarcinoma cell signaling”. All genes were filtered by relevance score >10, and protein-coding genes only.

2.2. Transcriptomic Validation

To confirm that the genes obtained from the GeneCards database are actually dysregulated in real cholangiocarcinoma patient tissues compared to normal controls, the validation was performed using the transcriptomic data from the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) Database. The GSE26566, microarray dataset (Molecular classification of human cholangiocarcinoma) from the GEO, which was deposited by Andersen J.B. and Thorgeirsson S.S., as described in the original article, was used. The dataset comprised 104 CCA tumor tissue samples, 59 surrounding liver tissue samples, and 6 normal intrahepatic bile duct samples. DEGs analysis was conducted using GEO2R, with CCA tumor tissues designated as the test group and normal intrahepatic bile duct tissues as the control group. To account for multiple testing, p-values were adjusted using the Benjamini–Hochberg method. Genes were considered significantly differentially expressed when the adjusted p-value was <0.05, with genes showing a log2 fold change (log2FC)>1 classified as upregulated and those with a log2FC <-1 classified as downregulated genes.

The volcano plot was used to visualize statistically significantly differentially expressed genes (DEGs), where the x-axis shows the log2FC, and the y-axis shows -log10 (*p*-value adjusted). The top 250 DEG based on the *p*-value, which also included the upregulated genes, were intersected with the CCA genes from GeneCards using Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/). Prior to intersection analysis, gene symbols from the GEO and GeneCards datasets were standardized to ensure consistency. As GEO2R outputs were already provided in gene symbols, standardization was performed using the HUGO Gene Nomenclature Committee (HGNC) database (https://www.genenames.org/) in Microsoft Excel to correct discrepancies due to aliases, outdated nomenclature, or case sensitivity. Duplicate genes were removed in Excel.

2.3. Protein-Protein Interaction

The STRING database was used to create and display the PPI network. The shared genes from GEO and GeneCards were submitted to the STRING 12 (https://string-db.org/) database, with "Homo sapiens" chosen as the species and employing a high confidence level of 0.700. The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted on the entire set of 12 gene nodes obtained from PPI using STRING. The ShinyGO online tools version 0.85.1 (https://bioinformatics.sdstate.edu/go/) were utilized to identify GO, which includes molecular function (MF), cellular component (CC), and biological process (BP), and the KEGG was used for the enrichment analysis of hub network genes. Statistical significance for the enrichment analysis was assessed using a threshold of *p*-value <0.05.

2.4. Hub Gene Identification

Cytoscape 3.10.2 was used to visualize the PPI network obtained from the STRING database. This software platform is developed for the visualization, analysis, and interpretation of complex biological systems and molecular interaction information. CytoHubba, a Cytoscape plugin, was used to identify hub genes in the established PPI network using multiple metrics. The metrics used in this study were MCC, MNC, Degree, and closeness. The ten hub genes exhibiting the shortest pathways were identified, and the highest-scoring genes in the most metrics were selected as therapeutic targets for the molecular docking analysis.

2.5. Phytochemicals Data

To explore phytochemicals that can bind to potential protein targets from the hub genes, we extensively searched the KNApSAcK World database (https://www.knapsackfamily.com) for Indonesian medicinal plants widely used in *jamu*. The search by country ("Indonesia") was applied. It contains information on thousands of species, including medicinal plants used in the region, along with their associated phytochemicals. An additional literature-based phytochemical search was also conducted to confirm the quantities of the phytochemicals in each medicinal plant. The criteria used to select the phytochemical components were abundance and drug-likeness, based on the Lipinski Rules, using the web-based tool SwissADME (https://www.swissadme.ch/).

In addition to Lipinski's Rule of Five, pharmacokinetic parameters, including topological polar surface area (TPSA) and gastrointestinal (GI) absorption, predicted by SwissADME were also evaluated. Although most compounds met the recommended criteria, compounds with less favorable properties were still subjected to molecular docking to comprehensively assess their binding potential.

2.6. Molecular Docking

Molecular docking simulations were performed using a personal laptop with the following specifications: an AMD Ryzen 5 processor (4500U), 12 GB RAM, running on a 64-bit Windows 10 Pro operating system. The computational visualization was supported by a Radeon Graphics 2.38 GHz. The molecular docking was performed on a laptop. The AutoDock tools software was used to prepare the receptors and ligands for docking. Crystal structures of the CCNA2 receptor (PDB ID: 7QHL) were obtained from the Protein Data Bank (www.rcsb.org). The hydrogen polar and Kollman charges were added to the protein, and the protein was converted to pdbqt format. The ligand's 3D structure, which is the phytochemicals from the chosen medicinal plants, was obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/). All atoms were assigned Gasteiger charges, and nonpolar hydrogen bonds were eliminated. The default number of rotatable bonds for the ligand was determined. These were saved in PDBQT format. The grid box for CCNA2 was defined with X = 24.911, Y = -16.195, and Z = 37.495 axes, and a grid spacing of x = 22, y = 20, and z = 34 Å.

AutoDock Vina was used for molecular docking simulations to evaluate the interactions between therapeutic targets and their receptor binding sites. The docking method was validated by redocking the native ligand and calculating the RMSD. Molecular docking simulations were performed to predict the binding poses and interactions of flexible small-molecule ligands within the binding site of a macromolecular receptor with a known three-dimensional structure. Compounds exhibiting more favorable binding affinity values (kcal/mol) were considered to have stronger predicted interactions with the receptor. The resulting docking poses and molecular interactions were subsequently analyzed and visualized in both 2D and 3D using Discovery Studio.

3. Results and Discussion

3.1. Gene Mining for Target Identification in Cholangiocarcinoma

The purpose of gene mining was to comprehensively identify candidate molecular targets associated with CCA pathogenesis, signaling, proliferation, and therapeutic resistance. These categories collectively represent the major hallmarks of cancer, including sustained proliferative signaling, evasion of apoptosis, and therapy resistance, which are critical in CCA progression [20]. Using the GeneCards database, four biologically relevant

categories were queried, yielding a total of 1251 non-redundant protein-coding genes after filtering (relevance score >10) as shown in Figure 1. The large number of genes obtained underscores the molecular complexity of CCA, which is known to involve dysregulation of multiple oncogenic pathways [21]. Importantly, integrating disease-specific and pathway-based gene sets increases the likelihood of identifying clinically relevant targets rather than isolated biomarkers, thereby strengthening the translational relevance for phytochemical intervention.

3.2. Transcriptomic Validation Using GEO Dataset (GSE26566)

The purpose of transcriptomic validation was to refine the candidate gene list by identifying genes that are not only theoretically associated with CCA but also significantly dysregulated in patient-derived datasets. Differential expression analysis using GEO2R on GSE26566 (CCA vs. normal intrahepatic bile duct) identified 807 DEGs, of which 786 genes were significant DEGs (p-value <0.05 and |log2FC>1|) as shown in the volcano plot in Figure 2A. From the top 250 DEG, 237 genes were included in the significant DEG, which consists of 135 upregulated and 102 downregulated genes after stringent filtering.

Intersecting the 135 significantly upregulated DEGs with the 1251 GeneCards-derived genes yielded 32 overlapping genes (Figure 2B), representing high-confidence targets. This integrative approach reduces false positives and highlights genes that are consistently overexpressed in tumor tissue, which are more suitable as therapeutic targets. The predominance of upregulated genes is particularly important for drug discovery, as these genes often encode proteins that drive oncogenic processes such as cell cycle progression, mitotic spindle formation, and DNA replication [22]. These processes are frequently hyperactivated in CCA and are associated with poor prognosis and chemoresistance.

3.3. Protein–Protein Interaction Network Analysis

The purpose of PPI analysis was to identify central hub proteins and functional modules among the validated targets, thereby prioritizing key molecular drivers for therapeutic intervention. Using STRING with a high-confidence threshold (0.700), the 32 candidate proteins were refined into a tightly connected network of 12 nodes (Figure 3), indicating strong functional interdependence. The resulting network highlights several critical hub proteins, including KIF11, CCNA2, CDK7, TOP2A, CDC20, TPX2, UBE2T, UHRF1, TACC3, E2F3, TYMS, and RAD51AP1. These proteins are predominantly involved in cell cycle regulation, mitotic spindle assembly, and DNA replication/repair [23, 24]. For instance, KIF11 is essential for bipolar spindle formation during mitosis, and its inhibition leads to mitotic arrest and apoptosis [25]. Similarly, CCNA2 and CDK7 regulate cell cycle transitions, particularly the G1/S and G2/M phases, which are frequently dysregulated in cancer [23].

The clustering of these proteins within a single network suggests that CCA progression is heavily dependent on coordinated activation of mitotic and proliferative machinery. This is consistent with previous findings that uncontrolled cell cycle progression is a hallmark of aggressive CCA phenotypes [26]. From a biomedical perspective, targeting such hub proteins offers a strategic advantage, as inhibition of a single node can disrupt multiple downstream oncogenic processes.

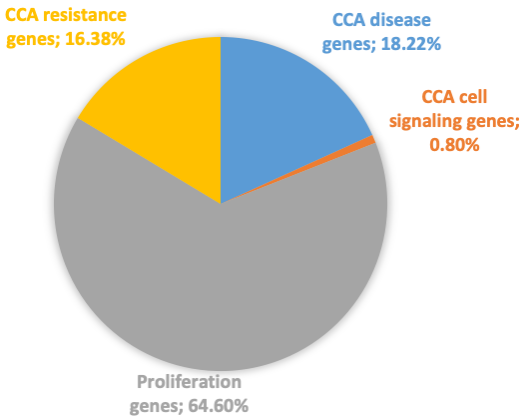


Figure 1. Distribution of CCA-associated genes identified through GeneCards-based gene mining across four functional categories: disease-related genes (18.22%), cell signaling genes (0.80%), proliferation-associated genes (64.60%), and resistance-related genes (16.38%). The analysis was performed using keyword-driven queries representing major oncogenic processes, followed by filtering for protein-coding genes with a relevance score >10

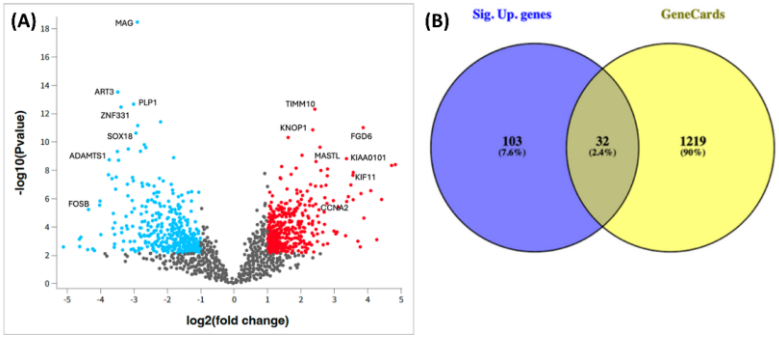


Figure 2. Integrated transcriptomic validation and gene prioritization for CCA target identification. (A) Volcano plot of DEG analysis derived from the GSE26566 dataset comparing cholangiocarcinoma tissues to normal intrahepatic bile duct samples. Significantly upregulated genes (red) and downregulated genes (blue) were defined using thresholds of $p < 0.05$ and $|\log2FC > 1|$. (B) Venn diagram of the intersection between significantly upregulated genes (n = 135) and GeneCards-derived cholangiocarcinoma-associated genes (n = 1251), resulting in 32 overlapping candidate genes

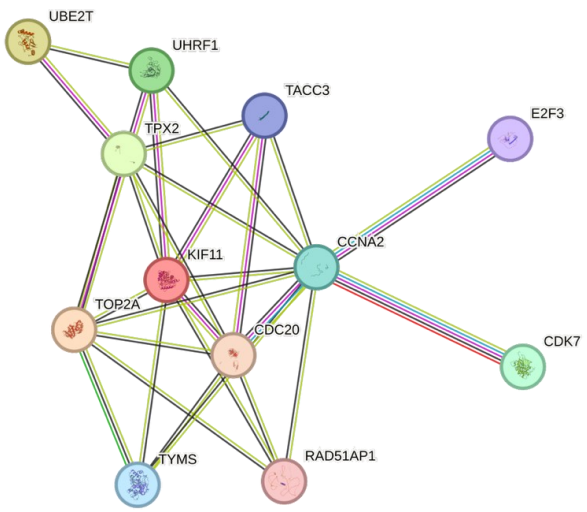


Figure 3. PPI network of 32 candidate proteins in STRING

3.4. Gene Ontology Analysis

GO analysis is a bioinformatics approach used to systematically classify genes into standardized functional categories based on the BP, CC, and MF domains. The principle of GO analysis is enrichment testing, in which a given gene set is statistically compared against a background genome to identify overrepresented biological terms, thereby revealing dominant functional themes [27]. Fold enrichment represents how much more frequently a specific GO term appears in the input gene set, suggesting a central and highly specific role in the disease mechanism. The number of genes reflects how many genes contribute to the same biological function.

The purpose of GO analysis in this study is to elucidate the biological roles and molecular mechanisms of the 12 candidate genes associated with CCA. This step directly supports the study objective by identifying critical biological pathways and molecular functions that can be targeted by Indonesian *jamu*-derived phytochemicals, particularly in the context of cancer proliferation, mitosis, and therapeutic resistance.

The GO-BP results (Figure 4A) demonstrate strong enrichment in processes related to mitotic spindle assembly, chromosome segregation, nuclear division, and cell cycle progression. The highest fold enrichment is observed in mitotic spindle assembly and mitotic spindle organization, indicating that the candidate genes are heavily involved in regulating mitosis. This finding is highly relevant to cholangiocarcinoma, as uncontrolled cell division is a hallmark of cancer, driven by dysregulation of mitotic machinery [20, 28]. Processes such as sister chromatid segregation and chromosome segregation are essential for genomic stability, and their disruption leads to chromosomal instability, a key feature of aggressive CCA [26]. Additionally, enrichment in DNA metabolic processes and cell cycle processes suggests that these genes contribute to rapid tumor cell proliferation. This supports the earlier GeneCards result, in which proliferation-associated genes constituted the largest proportion (64.60%), confirming that cell cycle dysregulation is a central driver of CCA pathogenesis.

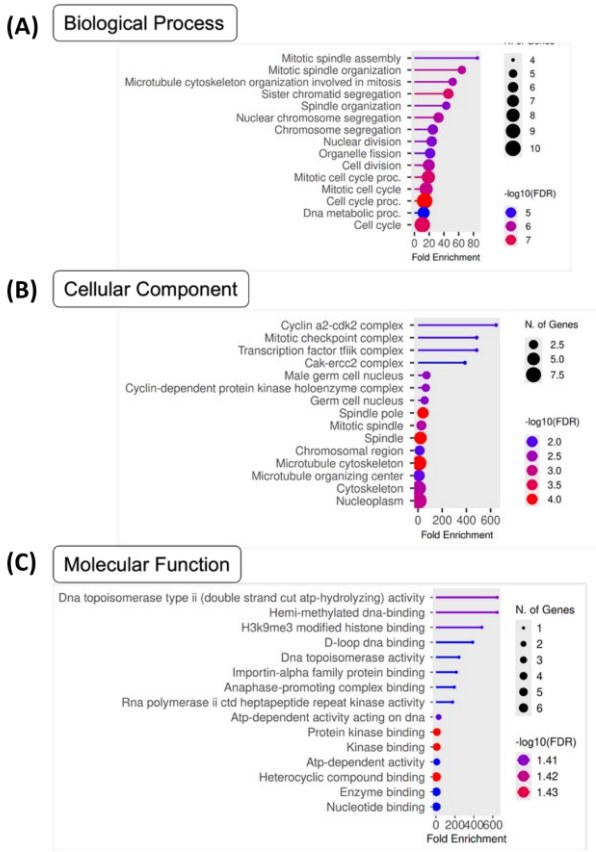


Figure 4. The bar plot of the GO enrichment analysis of the 12 connected genes associated with CCA

The GO-CC analysis (Figure 4B) reveals that the identified proteins are primarily localized in cyclin-CDK complexes, the mitotic spindle, the spindle pole, the microtubule cytoskeleton, and the nucleoplasm. The enrichment of cyclin A2-CDK2 complex and CDK holoenzyme complex highlights the importance of cyclin-dependent kinase regulation in CCA. CDKs are central regulators of cell cycle transitions, and their overactivation is commonly observed in cancers, including cholangiocarcinoma [29]. Furthermore, localization to the mitotic spindle and microtubule-organizing centers reinforces the importance of cytoskeletal dynamics in tumor progression. Proteins such as KIF11 and TPX2, identified in the PPI network, are directly involved in spindle assembly and stabilization [30]. Disruption of these structures can impair chromosome segregation, leading to apoptosis. The presence of these proteins in the nucleoplasm and chromosomal regions also indicates their involvement in DNA replication and transcriptional regulation, further linking them to oncogenic transformation [31, 32].

The GO-MF results (Figure 4C) indicate enrichment in functions such as DNA topoisomerase activity, ATP-dependent DNA activity, kinase binding, and protein kinase binding. The enrichment of DNA topoisomerase type II activity is particularly significant, as TOP2A (identified in the PPI network) is a well-known target in cancer therapy. This enzyme is critical for DNA replication and chromosome segregation, and its inhibition leads to DNA damage and cell death [33]. Additionally, enrichment in kinase binding and protein

kinase binding suggests that many of these proteins interact with signaling pathways that regulate cell proliferation, such as CDK-mediated phosphorylation cascades. This supports the involvement of key oncogenic pathways (e.g., PI3K/AKT and MAPK) identified during gene mining. The presence of ATP-dependent activities acting on DNA further emphasizes the high metabolic and replicative demand of CCA cells, which can be exploited therapeutically by compounds that disrupt ATP-binding or enzymatic activity [34].

Collectively, the GO analysis reveals that the candidate genes are functionally convergent on mitotic regulation, cell cycle progression, and DNA metabolism, which are critical processes driving cholangiocarcinoma development. This convergence strongly supports the hypothesis that CCA is highly dependent on proliferative and mitotic machinery, making these pathways ideal targets for therapeutic intervention.

3.5. Kyoto Encyclopedia of Genes and Genomes (KEGG) Analysis

KEGG pathway enrichment analysis translates the GO functional signals into pathway-level mechanisms. In this study, KEGG analysis, which was performed on 12 hub genes from STRING PPI, reveals a strong convergence toward cell cycle–related mechanisms, which is highly consistent with the GO results (Figure 5). The cell cycle pathway exhibits the highest fold enrichment and statistical significance, indicating that a substantial proportion of these interconnected genes are directly involved in regulating mitotic progression and cellular proliferation. This finding reinforces the GO BP terms, including mitotic spindle organization, chromosome segregation, and overall cell cycle regulation, confirming that uncontrolled proliferation is the central driver of cholangiocarcinoma in this dataset [35]. Additionally, enrichment in pathways such as cellular senescence suggests a disruption in the balance between proliferation and growth arrest, where tumor cells may bypass senescence checkpoints while still retaining partial regulatory signaling [36].

Interestingly, several enriched pathways, including human T-cell leukemia virus 1 infection, Epstein–Barr virus infection, hepatitis B, and viral carcinogenesis, do not necessarily indicate viral involvement but rather reflect shared oncogenic signaling components [37], such as NF-κB activation, anti-apoptotic pathways, and cell cycle dysregulation. These pathways highlight the overlap between tumor biology and mechanisms commonly exploited during virus-induced transformation, further supporting the presence of a broad oncogenic network [38].

The KEGG enrichment results demonstrating strong involvement of the cell cycle pathway are consistent with GO findings and reflect a fundamental mechanism of tumorigenesis, as dysregulated cell cycle progression is a hallmark of cancer [39].

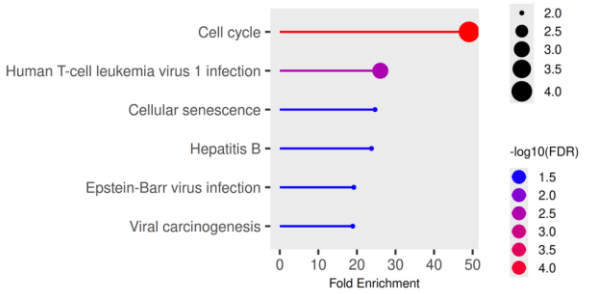


Figure 5. KEGG pathway enrichment analysis of 12 hub genes from STRING PPI. The dot plot shows significantly enriched pathways ranked by fold enrichment (x-axis), with dot size representing gene count and color indicating statistical significance (-log10 FDR)

3.6. Hub Gene Identification

The CytoHubba, a Cytoscape plugin, was used to analyze the PPI network and identify key hub genes using multiple topological algorithms [40]. In this study, the MCC, MNC, Degree, and Closeness centrality metrics were employed, providing a robust and comprehensive evaluation of node importance. Across all four metrics, a consistent pattern emerged in which CCNA2, KIF11, CDC20, and TOP2A were repeatedly ranked among the top nodes, indicating their strong centrality and critical regulatory roles within the network. In the MCC analysis, which is widely regarded as one of the most reliable methods for identifying essential proteins in biological networks, CCNA2 and KIF11 showed the highest scores, suggesting their involvement in highly interconnected functional modules (Figure 6A).

Similarly, MNC analysis highlighted KIF11 and CCNA2 as top-ranked genes, reflecting their importance within densely connected subnetworks (Figure 6B). Degree centrality further confirmed CCNA2 as the most connected node, followed by KIF11 and CDC20, indicating that these proteins interact with multiple partners and likely exert broad regulatory influence (Figure 6C). Closeness centrality again ranked CCNA2 and KIF11 at the top, suggesting their ability to efficiently communicate with other nodes and rapidly influence the entire network (Figure 6D) [41].

The consistency of these results across different algorithms strengthens the reliability of these genes as true hub genes rather than artifacts of a single metric. Functionally, the identified hubs are well-established regulators of the cell cycle and mitotic progression, which is in strong agreement with the GO and KEGG enrichment results. CCNA2 or cyclin A2 plays a crucial role in cell cycle phase transition, KIF11 is essential for mitotic spindle assembly, CDC20 regulates the anaphase-promoting complex, and TOP2A is required for DNA replication and chromosome segregation. These genes are frequently overexpressed and associated with poor prognosis in multiple cancers, further supporting their biological importance [42].

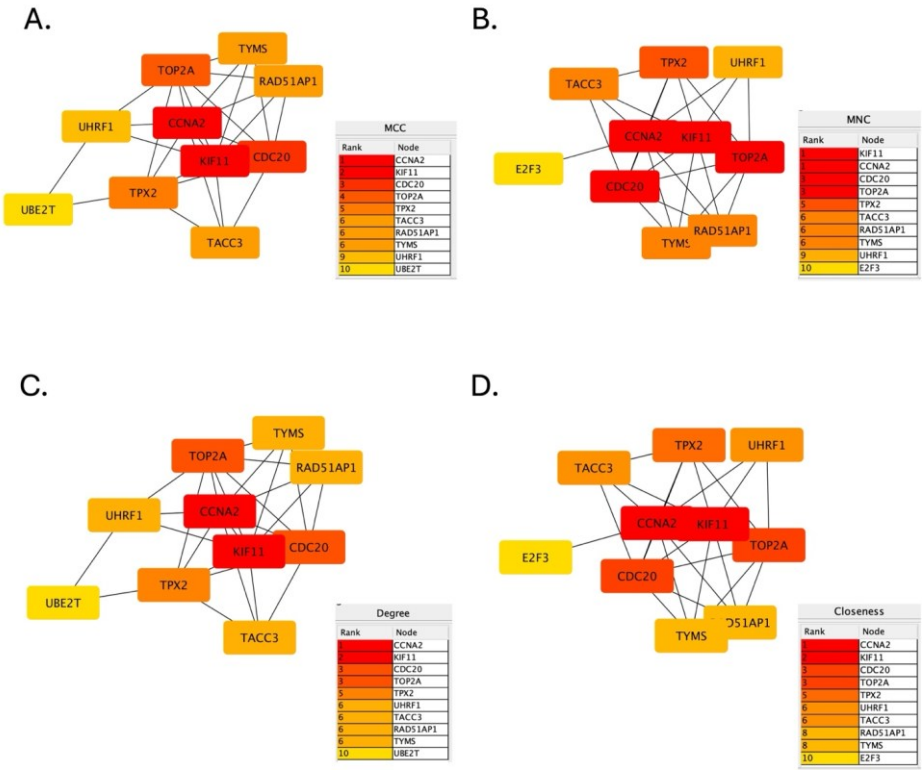


Figure 6. Identification of hub genes from the PPI network using the CytoHubba plugin in Cytoscape. Hub genes were ranked according to four topological algorithms: (A) Maximal Clique Centrality (MCC), (B) Maximum Neighborhood Component (MNC), (C) Degree, and (D) Closeness centrality. Nodes are colored according to their ranking scores

The convergence of results from PPI network analysis, GO, and KEGG enrichment provides multi-layer validation, increasing confidence that these genes are not only topologically central but also biologically and clinically significant. Several of these hub genes, particularly KIF11 and TOP2A, have already been explored as anticancer drug targets, highlighting the translational relevance of this approach [24, 25]. Collectively, the CytoHubba analysis identifies a core module of mitosis-related hub genes that represent promising candidates for further molecular docking and targeted therapeutic development.

CCNA2 was selected as the primary target for further molecular docking analysis. Biologically, CCNA2 encodes cyclin A2, a critical regulator of both S phase progression and the G2/M transition, making it a key driver of cell cycle control [43]. Compared to other hub genes such as KIF11 (primarily involved in mitotic spindle formation), CDC20 (a co-activator of the anaphase-promoting complex), and TOP2A (a DNA topology regulator), CCNA2 operates at multiple checkpoints of the cell cycle, providing a wider therapeutic intervention window. Moreover, CCNA2 dysregulation has been consistently associated with tumor aggressiveness, poor prognosis, and uncontrolled proliferation, reinforcing its clinical significance [31, 44]. From a drug discovery perspective, targeting such a central regulator increases the likelihood of disrupting coordinated cell cycle progression rather than a single mitotic event, thereby enhancing therapeutic efficacy.

3.7. Docking Method Validation

Prior to evaluating the binding affinity of test compounds, molecular docking protocols must be validated to ensure that the selected docking parameters can accurately reproduce the experimentally observed ligand-binding mode. Docking validation was performed through a re-docking procedure. The resulting docked pose is then compared with the experimentally determined crystallographic pose by calculating the Root Mean Square Deviation (RMSD) between the two conformations. RMSD measures the average distance between corresponding atoms of the crystallographic and predicted ligand conformations after optimal structural alignment. This validation step is essential because it assesses the reliability of the docking protocol in predicting ligand orientation and receptor–ligand interactions within the active site.

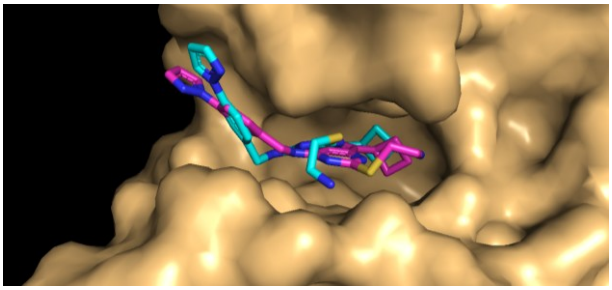


Figure 7. Molecular docking validation by re-docking the native ligand D5P into the CCNA2 binding site. Superimposition of the crystallographic ligand conformation (magenta) and the predicted docking pose (cyan) yielded an RMSD value of 2.584 Å

Figure 7 shows the superimposition of the crystallographic native ligand 5-(2-amino-1-ethyl)thio-3-cyclobutyl-7-[4-(pyrazol-1-yl)benzyl]amino-1(2)H-pyrazolo[4,3-d]pyrimidine (D5P) pose (magenta) and the re-docked pose (cyan) within the receptor cavity. Most of the central molecular scaffold occupies the same region of the binding pocket, with only minor deviations observed at the terminal portions of the ligand, indicating that the docking algorithm successfully reproduced the experimentally observed binding mode. Such deviations are commonly observed due to the intrinsic flexibility of ligand side chains and the rigid receptor approximation employed in docking protocols.

Lower RMSD values indicate better agreement between experimental and predicted binding poses. An RMSD value below 2.0 Å is generally considered excellent, whereas values of 2.0–3.0 Å are generally acceptable for docking validation. Values above 3.0 Å may indicate poor reproduction of the native binding mode [45]. The obtained RMSD value of 2.584 Å generally indicates a moderate validation range, indicating that the docking protocol possesses sufficient predictive capability to reproduce the experimentally observed binding orientation of D5P within the CCNA2 active site. Although the value slightly exceeds the ideal threshold of 2.0 Å, the overall overlap between the superimposed structures indicates that the key binding interactions and spatial positioning of the ligand are preserved. The acceptable RMSD value provides confidence that the predicted binding poses and interaction profiles are likely to reflect biologically relevant receptor–ligand interactions. Thus, the validated protocol was considered suitable for subsequent virtual screening and molecular docking of phytochemicals.

3.8. Phytochemicals Exploration from Indonesian-*Jamu* Medicinal Plants

The selection of the phytochemicals was rationally based on their ethnobotanical relevance, particularly their longstanding use as *jamu*, the traditional Indonesian herbal beverages widely consumed for maintaining health and treating various ailments [9]. Medicinal plants such as *C. longa*, *C. xanthorrhiza*, *Z. officinale*, *K. galanga, Z. zerumbet,* and *B. pandurata* are traditionally recognized for their anti-inflammatory, anticancer, and antimicrobial properties. Compounds including curcumin, demethoxycurcumin, bisdemethoxycurcumin, xanthorrhizol, α-curcumene, β-sesquiphellandrene, gingerol, and others were further screened based on drug-likeness criteria, particularly Lipinski's Rule of Five, ensuring favorable pharmacokinetic properties such as appropriate molecular weight, lipophilicity, hydrogen bond donors/acceptors, and oral bioavailability potential.

In addition to Lipinski's Rule of Five, most selected phytochemicals exhibited favorable TPSA values (≤140 Å) [46] and high predicted GI absorption in Table 1. Two compounds showed higher TPSA values (>140 Å) and low GI absorption. These compounds were still included in the molecular docking analysis to comprehensively assess their interaction potential with the target protein. However, the docking results showed unfavorable binding energies, supporting their lower prioritization. This approach ensures that compound selection was guided by both pharmacokinetic properties and structure-based binding evaluation.

The binding energy from the molecular docking results presented in Table 2 indicates the binding affinity of selected phytochemicals from ethnomedicinal plants toward the CCNA2 protein, where more negative binding energy values indicate stronger and more stable ligand–protein interactions. When compared to the native ligand, which exhibits a binding affinity of -8.8 kcal/mol, curcumenol, kaempferol, quercetin, and boesenbergin A exhibit stronger binding affinity to CCNA2, suggesting their potential to interfere with cyclin A2 function and consequently disrupt cell cycle progression. Several phytochemicals demonstrate comparable or slightly weaker interactions with the CCNA2 protein. Although most tested phytochemicals may not surpass the binding strength of the native ligand, their comparable affinities, combined with the natural origin, favorable drug-likeness, and pharmacokinetic properties, highlight their potential as safer alternative candidate inhibitors or lead compounds for further optimization.

To understand how the ligand binds within the active site of the CCNA2 protein at the atomic level, revealing the key amino acid residues involved, the types of intermolecular interactions formed, and the chemical forces that stabilize the ligand–protein complex, the visualization was performed using BioVia Discovery Studio. Figure 8 shows the interaction of native ligand D5P in the active site of CCNA2. As shown in Figure 8A, the ligand is well accommodated within the binding pocket of CCNA2, indicating a favorable spatial fit and proper orientation for interaction. The detailed 3D interaction in Figure 8B and the 2D schematic in Figure 8C highlight that the binding is predominantly stabilized by hydrogen bonding and hydrophobic interactions, which are critical for ligand affinity and specificity.

A conventional hydrogen bond is observed with Lys33, serving as a key polar interaction that anchors the ligand within the binding site and contributes significantly to binding specificity and stability [47]. Multiple π–sigma and π–alkyl interactions between the aromatic rings of the ligand and hydrophobic residues such as Ile10, Val18, Val64, Leu134, Ala31, and Ala144, reflecting strong van der Waals and hydrophobic contacts. These interactions arise from the electron-rich π systems of the ligand engaging with nonpolar side chains, which enhances binding through dispersion forces. The presence of alkyl interactions further supports the hydrophobic environment of the binding pocket, facilitating ligand stabilization by minimizing solvent exposure [48].

Table 1. The Indonesian *jamu* medicinal plant, selected phytochemicals, and binding affinity against the CCNA2 protein were obtained through molecular docking analysis

Medicinal plant	Phytochemical	Violation of Lipinski's rule	TPSA	GI adsorption	Binding energy (kcal/mol)
-	CCNA2 native ligand (D5P)	0	-	-	-8.8
Turmeric (*C. longa*) [49]	Curcumenol	0	29.46	High	-8.9
	Curcumin	0	93.06	High	-8.6
	Desmethoxycurcumin	0	83.83	High	-8.6
	Bisdemetoxycurcumin	0	74.60	High	-8.6
	α-Eudesmol	0	20.23	High	-8.7
Javanese turmeric (*C. xanthorrhiza*) [49]	Xanthorrhizol	0	17.07	High	-7.9
	ar-curcumene	0	0.00	Low	-7.8
	β-bisabolol	0	20.23	High	-7.2
	Germacrone	0	17.07	High	-8.4
	β-elemenen	0	17.07	High	-8.2
Ginger (*Z. officinale*) [50, 51]	6-gingerol	0	66.76	High	-7.1
	6-shogaol	0	46.53	High	-7.3
	Gingerdione	0	63.60	High	-7.1
	Zingerone	0	46.53	High	-6.9
	Paradol	0	46.53	High	-7.1
Aromatic ginger (*K. galanga*) [52]	Ethyl cinnamate	0	26.30	High	-7
	Kaempferol	0	-	-	-9
	Kaempferide	0	100.13	High	-8.1
	Galangin	0	90.90	High	-8.4
	Ethyl-p-methoxycinnamate	0	46.53	High	-6.4
Bitter ginger (*Z. zerumbet*) [53, 54]	Tectorigenin	0	100.13	High	-8.5
	Cisimaritin	0	89.13	High	-8.6
	Zerumbone	0	17.07	High	-8
	Kaempferol 3-O-rhamnoside	0	172.88	Low	-8.9
	Quercetin	0	131.36	High	-9.2
Finger root (*B. pandurata*) [55, 56]	Pinocembrin	0	66.76	High	-8.8
	Pinostrobin	0	55.76	High	-8.5
	5-O-methylnaringenin	0	75.99	High	-8.6
	Boesenbergin A	0	55.76	High	-9.3
	Sakuranetin	0	75.99	High	-8.5

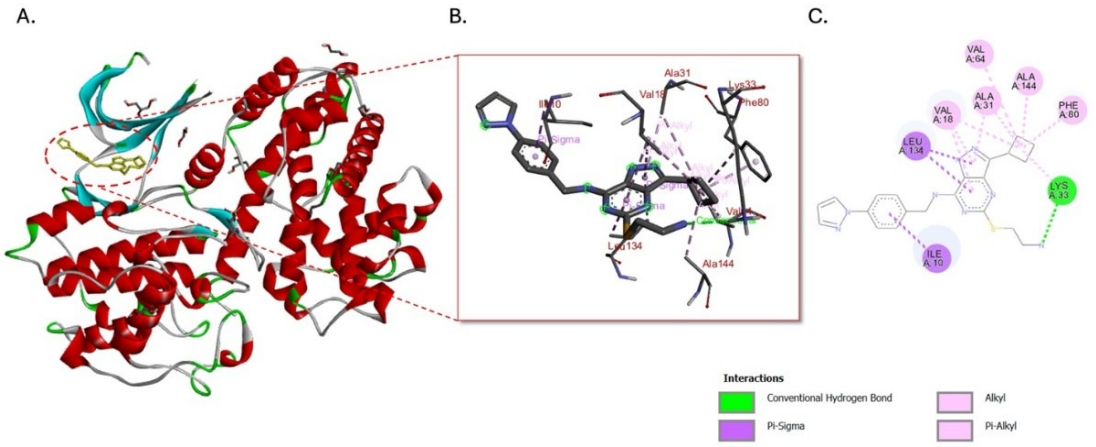


Figure 8. Molecular docking visualization of the native ligand (D5P) bound to the CCNA2 protein. (A) 3D structure of CCNA2 showing the ligand positioned within the active binding pocket. (B) Detailed 3D interaction illustrating key amino acid residues involved in ligand binding and the types of intermolecular interactions. (C) 2D interaction diagram

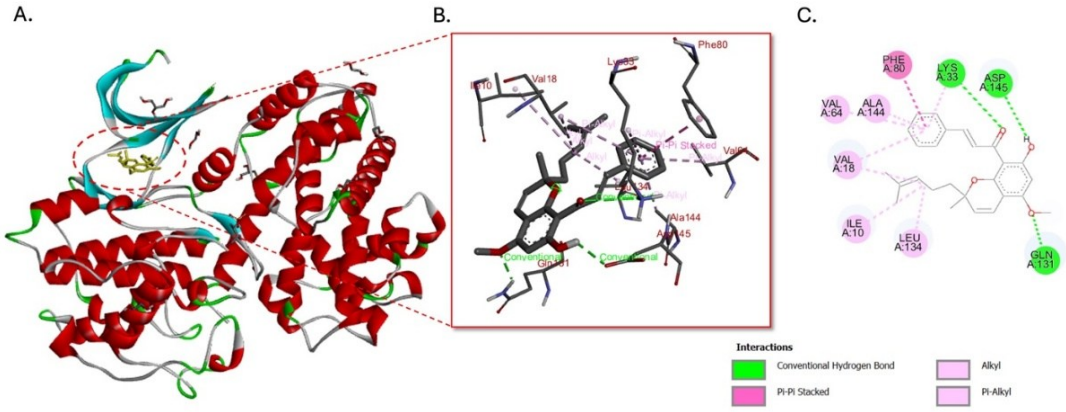


Figure 9. Molecular docking visualization of boesenbergin A bound to the CCNA2 protein. (A) 3D structure of CCNA2 showing the ligand positioned within the active binding pocket. (B) Detailed 3D interaction illustrating key amino acid residues involved in ligand binding and the types of intermolecular interactions. (C) 2D interaction diagram

Chemically, the combination of polar (hydrogen bonding) and nonpolar (π-based and alkyl) interactions indicates that the ligand achieves an optimal balance between specificity and binding strength, a hallmark of effective protein–ligand complexes. Overall, this interaction profile confirms that the native ligand is strongly stabilized within the CCNA2 binding pocket through cooperative non-covalent forces, providing a reliable reference model for evaluating and comparing the binding behavior of candidate phytochemicals.

Figure 9 shows the interaction between the lowest binding affinity phytochemical, boesenbergin A, a chalcone-derived bioactive compound from finger root (*B. pandurata*), and the cyclin A2 (CCNA2) receptor. The 3D docking pose (Figure 9A) demonstrates that boesenbergin A is well accommodated within the binding pocket of CCNA2. The ligand adopts a conformation that allows both its aromatic rings and oxygen-containing functional groups to interact favorably with the surrounding residues, suggesting that it is sterically compatible with the receptor cavity and capable of establishing multiple stabilizing interactions.

A closer examination of the binding site (Figure 9B) reveals that hydrogen bonding and hydrophobic interactions are the primary forces stabilizing the boesenbergin A and CCNA2 complex. Two conventional hydrogen bonds (green dashed lines) are formed between the oxygen atoms of boesenbergin A and residues Gln131 and Asp145. Hydrogen bonds are highly directional electrostatic interactions that play a critical role in ligand recognition and specificity. The carbonyl and ether oxygen atoms of boesenbergin A act as hydrogen-bond acceptors, interacting with hydrogen-bond donor groups from the amino acid side chains. These interactions help anchor the ligand within the binding pocket and significantly stabilize the complex [47].

Extensive hydrophobic interactions are observed at several residues, including Val18, Val64, Ile10, Leu134, Ala144, and Lys33, which participate in alkyl and π-alkyl interactions with the aromatic and aliphatic regions of boesenbergin A. Alkyl and π-alkyl interactions arise from favorable van der Waals contacts between hydrophobic amino acid side chains and the π-electron cloud of aromatic rings. A particularly notable interaction is the $\pi-\pi$ stacking between the aromatic ring of boesenbergin A and Phe80, enabling favorable interactions between delocalized π-electron clouds. This interaction is especially important because aromatic residues, such as phenylalanine, often contribute significantly to ligand stabilization within protein binding sites [57]. The presence of $\pi-\pi$ stacking suggests that the aromatic scaffold of boesenbergin A is a key structural feature responsible for its affinity toward CCNA2.

The 2D interaction diagram (Figure 9C) further confirms that the residues Val18, Val64, Ile10, Leu134, Ala144, and Phe80 create a hydrophobic environment that accommodates the phenyl rings and prenylated side chain of boesenbergin A. The hydrophobic nature of these interactions is chemically consistent with the structure of boesenbergin A, which contains multiple aromatic rings and nonpolar carbon chains. Meanwhile, the oxygen-containing functional groups remain oriented toward polar residues (Gln131 and Asp145), maximizing hydrogen-bond formation while preserving hydrophobic complementarity [58].

It is important to note that the molecular docking performed in this study assumes a rigid receptor conformation, which may not fully represent the dynamic nature of proteins under physiological conditions. In reality, ligand binding can induce conformational changes in the target protein (induced-fit effects), particularly in flexible regions involved in binding or protein–protein interactions, such as those present in CCNA2 [59]. Therefore, the predicted binding affinities may not completely reflect the true interaction dynamics. Incorporating protein flexibility through approaches such as molecular dynamics simulations would provide a more accurate assessment of ligand binding stability and interaction mechanisms [60].

Table 2. A summary of the chemical interaction types and the amino acid residue of phytochemicals with the lowest binding energy

Compound	Binding energy (kcal/mol)	Binding type	Amino acid residue
D5P	-8.8	Hydrogen bonding	LYS33
		Alkyl	ALA31, VAL64, PHE60, ALA144,
		π-Alkyl	VAL18, ALA31
		π-sigma	LEU134, ILE10
Boesenbergin A	-9.3	Hydrogen bonding	LYS33, ASP145, GLN131
		π-π stacked	PHE80
		Alkyl	LEU134, ILE10, VAL18
		π-Alkyl	VAL18, ALA144,VAL64
Quercetin	-9.2	Hydrogen bonding	LEU83, LYS33
		π-π T shape	PHE80
		π-Alkyl	ALA144, VAL18, VAL64, ALA31
		π-sigma	LEU134, ILE10
Kaempferol	-9	Hydrogen bonding	LEU83, GLU81, ASP86, GLU51
		π-Alkyl	ALA31,VAL18, ALA144, VAL64
		π-sigma	LEU134, ILE10

In terms of selectivity, flavonoids such as quercetin and chalcone derivatives, including boesenbergin A, share structural features that are well known for their polypharmacological behavior. This is attributed to their planar structure, hydroxyl groups, and ability to form hydrogen bonds, which enable binding to various kinases or non-kinase proteins. These compounds have been reported to modulate multiple signaling pathways, potentially enhancing their efficacy through multi-pathway inhibition. On the other hand, this broad-spectrum activity also suggests a likelihood of off-target binding and limited selectivity [61].

Unlike classical kinase targets, CCNA2 (cyclin A2) is a non-kinase regulatory protein that controls cell cycle progression through interactions with cyclin-dependent kinases (CDKs) rather than intrinsic enzymatic activity [62]. In the context of CCNA2, the observed binding affinity may therefore reflect the ability of these compounds to interact with protein–protein interaction interfaces, potentially influencing the formation or stability of CCNA2–CDK complexes. However, such interactions may not be highly specific. Therefore, their lack of target selectivity and potential off-target interactions should be considered. This is particularly relevant since CCNA2 is involved in tightly regulated cell cycle processes, and non-specific modulation could affect multiple pathways [63]. Further validation, such as molecular dynamics simulations, binding assays, or cell-based functional studies, is required to determine whether these compounds specifically disrupt CCNA2-related interactions or exert broader biological effects.

4. Conclusion

In conclusion, this study successfully identified CCNA2 as a key therapeutic target through integrative network pharmacology and protein–protein interaction analysis, consistently ranking as a top hub gene across multiple CytoHubba algorithms, indicating its central role in regulating cell cycle progression. Functional enrichment further supported this finding by highlighting the dominance of cell cycle–related pathways, reinforcing the biological relevance of CCNA2 as a strategic intervention point. Subsequent molecular docking analysis demonstrated that selected phytochemicals derived from Indonesian *jamu* medicinal plants exhibit favorable binding affinities toward CCNA2, with several compounds showing the lowest and others comparable to the native ligand, suggesting their potential to effectively interact with and inhibit the protein. The binding interactions were stabilized by a combination of hydrogen bonding and hydrophobic forces, reflecting the formation of a chemically favorable ligand–protein complex. Importantly, the selection of these compounds was grounded in ethnobotanical evidence and supported by drug-likeness screening using Lipinski's Rule of Five, thereby strengthening their feasibility as orally active therapeutic candidates. Overall, this study highlights the promise of Indonesian *jamu* phytochemicals as potential CCNA2 inhibitors and provides a strong computational foundation for their further development as novel therapeutic agents targeting cell-cycle dysregulation. To bridge these computational predictions with experimental validation, immediate forward-looking in vitro studies are warranted. First, direct target engagement and binding kinetics should be quantified using cell-free recombinant CCNA2/CDK2 kinase inhibition assays to determine exact IC_{50} values. Second, phenotypic validation should be performed on human cholangiocarcinoma cell lines to measure cytotoxicity and cell-cycle arrest specifically at S and G2/M phases via flow cytometry. Third, an animal model will further confirm target-specific tumor suppression in vivo. These experimental steps will provide critical evidence to establish Indonesian *jamu* phytochemicals as viable drug leads against CCNA2-driven cholangiocarcinoma.

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References

[1] Baiq Sheila Okthalia Hakim, Fadila Rahmawati, Alana Akmal Yuar, Febi Jian Setiasari, Nafisya Ayu Evana, Moh Rizky Yolanda B. S., Diagnosis and Management of Cholangiocarcinoma (CCA), *Jurnal Biologi Tropis*, 25, 4, (2025), 4810-4820 https://doi.org/10.29303/jbt.v25i4.10162

[2] Nittaya Chamadol, Vallop Laopaiboon, Apiwat Jareanrat, Vasin Thanasukarn, Tharatip Srisuk, Vor Luvira, Poowanai Sarkhampee, Winai Ungpinitpong, Phummarat Khamvijite, Yutthapong Chumnanua, Nipath Nethuwakul, Passakorn Sodarat, Samrit Thammarit, Anchalee Techasen, Jaruwan Thuanman, Chaiwat Tawarungruang, Bandit Thinkhamrop, Prakasit Sa-Ngiamwibool, Watcharin Loilome, Piya Prajumwongs, Attapol Titapun, Improvement of survival outcomes of cholangiocarcinoma by ultrasonography surveillance: Multicenter retrospective cohorts, *Heliyon*, 10, 18, (2024), e38191 https://doi.org/10.1016/j.heliyon.2024.e38191

[3] Xuan Li, Renchu Guan, Shuangquan Zhang, Factors Contributing to the High Malignancy Level of Cholangiocarcinoma and Its Epidemiology: Literature Review and Data, *Biology*, 14, 4, (2025), 351 https://doi.org/10.3390/biology14040351

[4] Chaiwat Tawarungruang, Narong Khuntikeo, Nittaya Chamadol, Vallop Laopaiboon, Jaruwan Thuanman, Kavin Thinkhamrop, Matthew Kelly, Bandit Thinkhamrop, Survival after surgery among patients with cholangiocarcinoma in Northeast Thailand according to anatomical and morphological classification, *BMC Cancer*, 21, (2021), 497 https://doi.org/10.1186/s12885-021-08247-z

[5] Thomas Hughes, Thomas O'Connor, Anchalee Techasen, Nisana Namwat, Watcharin Loilome, Ross H. Andrews, Narong Khuntikeo, Puangrat Yongvanit, Paiboon Sithithaworn, Simon D. Taylor-Robinson, Opisthorchiasis and cholangiocarcinoma in Southeast Asia: an unresolved problem, *International Journal of General Medicine*, 10, (2017), 227-237 https://doi.org/10.2147/IJGM.S133292

[6] Qingliang Jiang, Xianglin Liu, Weichen Yu, Yangyang Li, Jiaqi Du, Xiaoqing Jiang, Hengyu Li, Integrated analysis reveals survival-related genes promoting cholangiocarcinoma progression via G2/M cell cycle, with validation of cyclin B1 in tumor proliferation, *Scientific Reports*, 15, (2025), 41352 https://doi.org/10.1038/s41598-025-25299-4

[7] P. Menapree, N. Duangthim, A. Sae-Fung, S. Sonkaew, S. Jitkaew, CDK4/6 inhibitors upregulate cIAP1/2, and Smac mimetic LCL161 enhances their antitumor effects in cholangiocarcinoma cells, *Scientific Reports*, 15, (2025), 6826 https://doi.org/10.1038/s41598-025-90997-y

[8] Tiza W. Mawaddah, Elisa D. Pratiwi, Astria N. Nidom, Jonny Jonny, Z. I. Ayyuba, I. Y. Rosytania, S. Indrasari, R. V. Nidon, C. A. Nidom, Towards Rigorous Safety Standards for Indonesian Jamu: A Review and Rationale for Non-Human Primate Toxicology Models, *Advances in Animal and Veterinary Sciences*, 14, 2, (2026), 342-355 https://doi.org/10.17582/journal.aavs/2026/14.2.342.355

[9] Waras Nurcholis, Rini Arianti, Jamu as Indonesian Cultural Heritage and Modern Health Innovation, *Jurnal Jamu Indonesia*, 9, 1, (2024), 1-2 https://doi.org/10.29244/jji.v9i1.317

[10] Maima Matin, Rajeev K. Singla, Artur Jóźwik, Jarosław Olav Horbańczuk, Natalia Ksepka, Kamil Wysocki, Thadiyan Parambil Ijinu, Neenthamadathil Mohandas Krishnakumar, Sreejith Pongillyathundiyil Sasidharan, Ifeoma C. Ezenyi, John Igoli, Fabio Fusi, Sara Frazzini, Luciana Rossi, Michel-Edwar Mickael, Abhishek Joshi, Olga Adamska, Artur Stolarczyk, Esra Capanoglu, Deniz Gunal-Koroglu, Shi-Hui Cheng, Omar M. Atrooz, Kiran Kharat, Ibrahim M. Abu-Reidah, Neeraj Rani, Atul Kabra, Ruchika Kabra, Dama Sreedhar Preethidan, Prathyusha Surendran, Emad Mohamed Abdallah, Seetha Harilal, Rajesh Kumar, Syed Abidullah, Hemanth Kumar Boyina, Vimal Arora, Prasanna Srinivasan Ramalingam, Sujatha Elangovan, Sivakumar Arumugam, Tanveer Alam, Edlira Aruci, Elena González-Burgos, Isabel Ureña-Vacas, Visitación López-Miranda, Esperanza Herradón, Rupesh Kumar Gautam, Rajat Goyal, Shah Alam Khan, Logesh Rajan, Joel Ojogbane Onoja, Sharad Vats, Akinleye Akinrinde, Smith B. Babiaka, Conrad V. Simoben, Doris E. Enow, Kennedy O. Abuga, Priti Talwar, Palaniyandi Ravanan, Reda El Boukhari, Ahmed Fatimi, Fabien Schultz, Ren-You Gan, Jean Noël Nyemb, Gaetan Bayiha Ba Njock, Constant Anatole Pieme, Goh Goh Bey Hing, Ricardo Lagoa, Nikolay T. Tzvetkov, Farhan Bin Matin, Antonello Santini, Jaya Arora, Aswathy Chankaramkandath Vasu, Suraj Kadunganattil, Abeer Essam Noman, Luay M. Alsubhi, Indra Lasmana Tarigan, Shafaat Yar Khan, Ali Zarrabi, Hefa Mangzira Kemung, Kavitha Raj Varadaraju, Tomasz M. Karpiński, Md. Mohaiminul Islam, Shaikh Jamal Uddin, Carmela Fimognari, Hari Prasad Devkota, Ivana Carev, Dongdong Wang, Kenneth Anchang Yongabi, Luther Bob Mbeku, Sohini Chakraborty, Sourav S. Patnaik, Shanmugam Thangapandiyan, Bikash Baral, Siva Sai Chandragiri, Eliana B. Souto, Gérard Lizard, Fatiha Brahmi, Farid Khallouki, Adil El Midaoui, Ronan Lordan, Anupam Bishayee, Meng-Yao Li, Monika Szymańska-Czerwińska, Krzysztof Niemczuk, Manik Chandra Shill, Michał Ławiński, Oleh Lushchak, Iwona Wojtasik-Kalinowska, Agnieszka Wierzbicka, Thomas Jakschitz, Mathew Dan, Imen Ghzaiel, Leila Rezig, Anne Vejux, Amira Zarrouk, Ahmad Ali, Andy Wai Kan Yeung, Günther K. Bonn, Bairong Shen, Atanas G. Atanasov, Health-promoting and medicinal properties of Zingiberaceae family plants: A minireview with a special focus on galangal, turmeric, cardamom, and ginger, *Current Research in Biotechnology*, 10, (2025), 100329 https://doi.org/10.1016/j.crbiot.2025.100329

[11] Sirinya Moakmamern, Lapamas Rueankham, Natsima Viriyaadhammaa, Wenxian Yin, Toyonobu Usuki, Suwit Duangmano, Yupanun Wutti-In, Sawitree Chiampanichayakul, Nutjeera Intasai, Siriporn Okonogi, Singkome Tima, Songyot Anuchapreeda, 6-Shogaol from *Zingiber officinale* Induces Cell Cycle Arrest via Suppression of c-Myc Protein Expression and Promotes Apoptosis in Human B-Cell Lymphoma, *International Journal of Molecular Sciences*, 27, 10, (2026), 4168 https://doi.org/10.3390/ijms27104168

[12] W. Park, A. R. M. R. Amin, Z. G. Chen, D. M. Shin, New Perspectives of Curcumin in Cancer Prevention, *Cancer Prevention Research*, 6, 5, (2013), 387-400 https://doi.org/10.1158/1940-6207.CAPR-12-0410

[13] A. Simamora, K. H. Timotius, M. B. Yerer, H. Setiawan, A. Mun'im, Xanthorrhizol, a potential anticancer agent, from *Curcuma xanthorrhiza* Roxb, *Phytomedicine*, 105, (2022), 154359 https://doi.org/10.1016/j.phymed.2022.154359

[14] Yingzi Wu, Jinhai Luo, Baojun Xu, Insights into the anticancer effects of galangal and galangin: A comprehensive review, *Phytomedicine*, 135, (2024), 156085 https://doi.org/10.1016/j.phymed.2024.156085

[15] S. Hu, Y. Xu, L. Meng, L. Huang, H. Sun, Curcumin inhibits proliferation and promotes apoptosis of breast cancer cells, *Experimental and Therapeutic Medicine*, 16, 2, (2018), 1266-1272 https://doi.org/10.3892/etm.2018.6345

[16] Nathan M. Weir, Karuppaiyah Selvendiran, Vijay Kumar Kutala, Liyue Tong, Shilpa Vishwanath, Murugesan Rajaram, Susheela Tridandapani, Shrikant Anant, Periannan Kuppusamy, Curcumin induces G2/M arrest and apoptosis in cisplatin-resistant human ovarian cancer cells by modulating akt and p38 mAPK, *Cancer Biology & Therapy*, 6, 2, (2007), 178-184 https://doi.org/10.4161/cbt.6.2.3577

[17] Mahmoud Hashemzaei, Amin Delarami Far, Arezoo Yari, Reza Entezari Heravi, Kaveh Tabrizian, Seyed Mohammad Taghdisi, Sarvenaz Ekhtiari Sadegh, Konstantinos Tsarouhas, Dimitrios Kouretas, George Tzanakakis, Dragana Nikitovic, Nikita Yurevich Anisimov, Demetrios A. Spandidos, Aristides M. Tsatsakis, Ramin Rezaee, Anticancer and apoptosis-inducing effects of quercetin in vitro and in vivo, *Oncology Reports*, 38, 2, (2017), 819-828 https://doi.org/10.3892/or.2017.5766

[18] Ahmad Almatroudi, Khaled S. Allemailem, Wanian M. Alwanian, Basmah F. Alharbi, Faris Alrumaihi, Amjad Ali Khan, Saleh A. Almatroodi, Arshad Husain Rahmani, Effects and Mechanisms of Kaempferol in the Management of Cancers through Modulation of Inflammation and Signal Transduction Pathways, *International Journal of Molecular Sciences*, 24, 10, (2023), 8630 https://doi.org/10.3390/ijms24108630

[19] Kuan-Beng Ng, Ahmad Bustamam, Mohd Aspollah Sukari, Siddig Ibrahim Abdelwahab, Syam Mohan, Michael James Christopher Buckle, Behnam Kamalidehghan, Nabilah Muhammad Nadzri, Theebaa Anasamy, A Hamid A Hadi, Heshu Sulaiman Rahman, Induction of selective cytotoxicity and apoptosis in human T4-lymphoblastoid cell line (CEMss) by boesenbergin a isolated from *boesenbergia rotunda* rhizomes involves mitochondrial pathway, activation of caspase 3 and G2/M phase cell cycle arrest, *BMC Complementary and Alternative Medicine*, 13, (2013), 41 https://doi.org/10.1186/1472-6882-13-41

[20] Douglas Hanahan, Hallmarks of Cancer: New Dimensions, *Cancer Discovery*, 12, 1, (2022), 31-46 https://doi.org/10.1158/2159-8290.CD-21-1059

[21] Giammarco Fava, Molecular mechanisms of cholangiocarcinoma, *World Journal of Gastrointestinal Pathophysiology*, 1, 1, (2010), 12-22 https://doi.org/10.4291/wjgp.v1.i1.12

[22] Özgür Cem Erkin, Betül Cömertpay, Esra Göv, Integrative Analysis for Identification of Therapeutic Targets and Prognostic Signatures in Non-Small Cell Lung Cancer, *Bioinformatics and Biology Insights*, 16, (2022), https://doi.org/10.1177/11779322221088796

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

[24] Tingting Shen, Lei Sheng, Hua Zhan, Sihan Chen, The analysis of TOP2A as a potential target for predicting the prognosis of brain glioma in the TCGA database and in single-center study, *Discover Oncology*, 16, (2025), 2291 https://doi.org/10.1007/s12672-025-04051-4

[25] Rand Shahin, Salah Aljamal, Kinesin Spindle Protein Inhibitors in Cancer: From High Throughput Screening to Novel Therapeutic Strategies, *Future Science OA*, 8, 3, (2022), FSO778 https://doi.org/10.2144/fsoa-2021-0116

[26] Alphonse E. Sirica, Gregory J. Gores, John D. Groopman, Florin M. Selaru, Mario Strazzabosco, Xin Wei Wang, Andrew X. Zhu, Intrahepatic Cholangiocarcinoma: Continuing Challenges and Translational Advances, *Hepatology*, 69, 4, (2019), 1803-1815 https://doi.org/10.1002/hep.30289

[27] The Gene Ontology Consortium, The Gene Ontology knowledgebase in 2026, *Nucleic Acids Research*, 54, D1, (2026), D1779-D1792 https://doi.org/10.1093/nar/gkaf1292

[28] Xiwei Ding, Tianlu Huang, Chunyan Peng, Keun Soo Ahn, Jesper B. Andersen, Monika Lewinska, Yu Cao, Guifang Xu, Gang Chen, Bo Kong, Helmut Friess, Shanshan Shen, Lewis R. Roberts, Lei Wang, Xiaoping Zou, Therapeutic Rationale to Target Highly Expressed Aurora kinase A Conferring Poor Prognosis in Cholangiocarcinoma, *Journal of Cancer*, 11, 8, (2020), 2241-2251 https://doi.org/10.7150/jca.31989

[29] Minako Yamamura, Yasunori Sato, Kenta Takahashi, Motoko Sasaki, Kenichi Harada, The cyclin-dependent kinase pathway involving CDK1 is a potential therapeutic target for cholangiocarcinoma, *Oncology Reports*, 43, 1, (2019), 306-317 https://doi.org/10.3892/or.2019.7405

[30] O. I. Kahn, N. Ha, M. A. Baird, M. W. Davidson, P. W. Baas, TPX 2 regulates neuronal morphology through kinesin-5 interaction, *Cytoskeleton*, 72, 7, (2015), 340-348 https://doi.org/10.1002/cm.21234

[31] W. Gao, J. Lu, Z. Yang, E. Li, Y. Cao, L. Xie, Mitotic Functions and Characters of KIF11 in Cancers, *Biomolecules*, 14, 4, (2024), 386 https://doi.org/10.3390/biom14040386

[32] J. Stumpff, P. N. Ghule, A. Shimamura, J. L. Stein, M. Greenblatt, Spindle Microtubule Dysfunction and Cancer Predisposition, *Journal of Cellular Physiology*, 229, 12, (2014), 1881-1883 https://doi.org/10.1002/jcp.24691

[33] P. J. Smith, S. R. McKeown, L. H. Patterson, Targeting DNA topoisomerase IIα (TOP2A) in the hypoxic tumour microenvironment using unidirectional hypoxia-activated prodrugs (UHAPS), *IUBMB Life*, 75, 1, (2023), 40-54 https://doi.org/10.1002/iub.2619

[34] K. Maemura, S. Natsugoe, S. Takao, Molecular mechanism of cholangiocarcinoma carcinogenesis, *Journal of Hepato-Biliary-Pancreatic Sciences*, 21, 10, (2014), 754-760 https://doi.org/10.1002/jhbp.126

[35] Zhixiang Wang, Regulation of Cell Cycle Progression by Growth Factor-Induced Cell Signaling, *Cells*, 10, 12, (2021), 3327 https://doi.org/10.3390/cells10123327

[36] Jing Liu, Yunhua Peng, Wenyi Wei, Cell cycle on the crossroad of tumorigenesis and cancer therapy, *Trends in Cell Biology*, 32, 1, (2022), 30-44 https://doi.org/10.1016/j.tcb.2021.07.001

[37] Yuichiro Hatano, Takayasu Ideta, Akihiro Hirata, Kayoko Hatano, Hiroyuki Tomita, Hideshi Okada, Masahito Shimizu, Takuji Tanaka, Akira Hara, Virus-Driven Carcinogenesis, *Cancers*, 13, 11, (2021), 2625 https://doi.org/10.3390/cancers13112625

[38] J. Charostad, M. Nakhaie, A. Dehghani, E. Faghihloo, The interplay between EBV and KSHV viral products and NF-κB pathway in oncogenesis, *Infectious Agents and Cancer*, 15, (2020), 62 https://doi.org/10.1186/s13027-020-00317-4

[39] H. K. Matthews, C. Bertoli, R. A. M. De Bruin, Cell cycle control in cancer, *Nature Reviews Molecular Cell Biology*, 23, (2022), 74-88 https://doi.org/10.1038/s41580-021-00404-3

[40] C. H. Chin, S. H. Chen, H. H. Wu, C. W. Ho, M. T. Ko, C. Y. Lin, *cytoHubba*: identifying hub objects and sub-networks from complex interactome, *BMC Systems Biology*, 8, (2014), S11 https://doi.org/10.1186/1752-0509-8-S4-S11

[41] A. L. Barabási, N. Gulbahce, J. Loscalzo, Network medicine: a network-based approach to human disease, *Nature Reviews Genetics*, 12, (2011), 56-68 https://doi.org/10.1038/nrg2918

[42] Cheerapinya Taebprakhon, Supannika Sorin, Kullanat Khawkhiaw, Chatchai Phoomak, Wunchana Seubwai, Sopit Wongkham, Charupong Saengboonmee, Pro-Tumorigenic Roles of Cyclin Dependent Kinase 2 and its Associated Cyclins in Cholangiocarcinoma Progression under High Glucose Condition, *Archives of Medical Research*, 57, 3, (2026), 103383 https://doi.org/10.1016/j.arcmed.2026.103383

[43] Ilenia Pellarin, Alessandra Dall'Acqua, Andrea Favero, Ilenia Segatto, Valentina Rossi, Nicole Crestan, Javad Karimbayli, Barbara Belletti, Gustavo Baldassarre, Cyclin-dependent protein kinases and cell cycle regulation in biology and disease, *Signal Transduction and Targeted Therapy*, 10, (2025), 11 https://doi.org/10.1038/s41392-024-02080-z

[44] Hiroyuki Yamano, APC/C: current understanding and future perspectives, *F1000Research*, 8, (2019), 725 https://doi.org/10.12688/f1000research.18582.1

[45] D. Ramírez, J. Caballero, Is It Reliable to Take the Molecular Docking Top Scoring Position as the Best Solution without Considering Available Structural Data?, *Molecules*, 23, 5, (2018), 1038 https://doi.org/10.3390/molecules23051038

[46] Daniel F. Veber, Stephen R. Johnson, Hung-Yuan Cheng, Brian R. Smith, Keith W. Ward, Kenneth D. Kopple, Molecular Properties That Influence the Oral Bioavailability of Drug Candidates, *Journal of Medicinal Chemistry*, 45, 12, (2002), 2615-2623 https://doi.org/10.1021/jm020017n

[47] Deliang Chen, Numan Oezguen, Petri Urvil, Colin Ferguson, Sara M. Dann, Tor C. Savidge, Regulation of protein-ligand binding affinity by hydrogen bond pairing, *Science Advances*, 2, 3, (2016), e1501240 https://doi.org/10.1126/sciadv.1501240

[48] R. Ferreira De Freitas, M. Schapira, A systematic analysis of atomic protein–ligand interactions in the PDB, *Medicinal Chemistry Communications*, 8, 10, (2017), 1970-1981 https://doi.org/10.1039/C7MD00381A

[49] Ibrahim Jantan, Fadlina Chany Saputri, Muhammad Naeem Qaisar, Fhataheya Buang, Correlation between Chemical Composition of *Curcuma domestica* and *Curcuma xanthorrhiza* and Their Antioxidant Effect on Human Low-Density Lipoprotein Oxidation, *Evidence-Based Complementary and Alternative Medicine*, 2012, (2012), 438356 https://doi.org/10.1155/2012/438356

[50] Romy Jon Hendra, Rusdi Rusdi, Ridho Asra, Sestry Misfadhila, Phytochemical and Traditional Uses of Red Ginger: A Review (*Zingiber officinale* var. rubrum), *EAS Journal of Pharmacy and Pharmacology*, 4, 3, (2022), 50-55 https://doi.org/10.36349/easjpp.2022.v04i03.002

[51] Swarupananda Mukjerjee, Dipanjan Karati, A mechanistic view on phytochemistry, pharmacognostic properties, and pharmacological activities of phytocompounds present in Zingiber officinale: A comprehensive review, *Pharmacological Research - Modern Chinese Medicine*, 5, (2022), 100173 https://doi.org/10.1016/j.prmcm.2022.100173

[52] Nataya Sutthanont, Wej Choochote, Benjawan Tuetun, Anuluck Junkum, Atchariya Jitpakdi, Udom Chaithong, Doungrat Riyong, Benjawan Pitasawat, Chemical composition and larvicidal activity of edible plant-derived essential oils against the pyrethroid-susceptible and -resistant strains of *Aedes aegypti* (Diptera: Culicidae), *Journal of Vector Ecology*, 35, 1, (2010), 106-115 https://doi.org/10.1111/j.1948-7134.2010.00066.x

[53] Tanat Na Nongkhai, Sarah E. Maddocks, Santi Phosri, Sarita Sangthong, Punyawatt Pintathong, Phanuphong Chaiwut, Kasemsiri Chandarajoti, Lutfun Nahar, Satyajit D. Sarker, Tinnakorn Theansungnoen, In Vitro Cytotoxicity and Antimicrobial Activity against Acne-Causing Bacteria and Phytochemical Analysis of Galangal (*Alpinia galanga*) and Bitter Ginger (*Zingiber zerumbet*) Extracts, *International Journal of Molecular Sciences*, 25, 20, (2024), 10869 https://doi.org/10.3390/ijms252010869

[54] Muhammad Ramzan, Basit Zeshan, Assessment of the Phytochemical Analysis and Antimicrobial Potentials of *Zingiber zerumbet*, *Molecules*, 28, 1, (2023), 409 https://doi.org/10.3390/molecules28010409

[55] Agus Chahyadi, Rika Hartati, Komar Ruslan Wirasutisna, Elfahmi Elfahmi, *Boesenbergia Pandurata* Roxb., An Indonesian Medicinal Plant: Phytochemistry, Biological Activity, Plant Biotechnology, *Procedia Chemistry*, 13, (2014), 13-37 https://doi.org/10.1016/j.proche.2014.12.003

[56] A. P. Widiyana, S. Herlina, R. Risadiansyah, G. Ashari, Major Phytochemicals of *Boesenbergia pandurata* Rhizome by LC-HRMS and Virtual Screening of *Staphylococcus aureus* Inhibition, *The 1st International Conference on Biological Technology and Engineering for Sustainable Nature (IC-BioTEStA 2025)*, 2026 https://doi.org/10.1051/bioconf/202620903008

[57] Esteban Lanzarotti, Rolf R. Biekofsky, Darío A. Estrin, Marcelo A. Marti, Adrián G. Turjanski, Aromatic–Aromatic Interactions in Proteins: Beyond the Dimer, *Journal of Chemical Information and Modeling*, 51, 7, (2011), 1623-1633 https://doi.org/10.1021/ci200062e

[58] R. Patil, S. Das, A. Stanley, L. Yadav, A. Sudhakar, A. K. Varma, Optimized Hydrophobic Interactions and Hydrogen Bonding at the Target-Ligand Interface Leads the Pathways of Drug-Designing, *PLoS ONE*, 5, 8, (2010), e12029 https://doi.org/10.1371/journal.pone.0012029

[59] Enrico Di Cera, Mechanisms of ligand binding, *Biophysics Reviews*, 1, (2020), 011303 https://doi.org/10.1063/5.0020997

[60] A. F. A. Hidayat, S. B. Mohamad, Incorporating Target Protein Structure Flexibility and Dynamics in Computational Drug Discovery Using Ensemble-Based Docking Analysis, *Journal of Visualized Experiments*, 220, (2025), 67174 https://doi.org/10.3791/67174

[61] J. Kubiak, P. Szyk, B. Czarczynska-Goslinska, T. Goslinski, Flavonoids, Chalcones, and Their Fluorinated Derivatives—Recent Advances in Synthesis and Potential Medical Applications, *Molecules*, 30, 11, (2025), 2395 https://doi.org/10.3390/molecules30112395

[62] Y. Gan, Y. Li, T. Li, G. Shu, G. Yin, CCNA2 acts as a novel biomarker in regulating the growth and apoptosis of colorectal cancer, *Cancer Management and Research*, 10, (2018), 5113-5124 https://doi.org/10.2147/CMAR.S176833

[63] Qing Wan, Zhongmin Yang, Lian Huang, Yang Xia, Lihua Long, Zucai Xu, Jida Li, Tumor cell cycle regulation: integrated perspective of stage characteristics, regulatory networks, and signaling pathway intervention strategies, *Molecular Biomedicine*, 7, (2026), 14 https://doi.org/10.1186/s43556-026-00411-w