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Short Communication

Beetroot (*Beta vulgaris*) potential in preventing colorectal cancer using in-silico analysis

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Abstract

Colorectal cancer (CRC) is one of the leading causes of cancer-related mortality worldwide, necessitating the need for an effective therapeutic strategy. *Beta vulgaris* (beetroot) possesses active compounds that exert anti-cancer properties. The aim of this study was to evaluate the potential of beetroot as a preventative agent against the progression of CRC using differentially expressed gene (DEG) analysis and network pharmacology approaches. The protein-protein interaction network and molecular docking analyses were employed to assess the key interactions of beetroot active compounds with CRC-related target protein. Cytotoxicity of beetroot extract was experimentally evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) assay on the HT29 cell line. The result of this study showed that protein in the cell cycle was significantly enriched in CRC, with *cyclin-dependent kinase 4* (*CDK4*) gene as one of the specific genes. Quercetin, galangin, hesperidin, farrerol, and betanin were the most typical compounds of beetroot based on the Comparative Toxicogenomics Database (CTD). Molecular docking studies revealed the strong binding affinity between quercetin (-7.04 kcal/mol) and bentanin (-8.11 kcal/mol) with CDK4. Beetroot demonstrated anticancer properties against the HT29 cell line with IC_{50} value of 39.03±1.4 µg/mL. In conclusion, the beetroot extract has inhibitory activity against HT29 cell line proliferation, highlighting its potential in preventing the development of CRC through the substantial suppression of gene expression within the cell cycle pathway.

Keywords: Ulcerative colitis, colorectal cancer, beet root (*Beta vulgaris*), CDK4, DEG analysis

Introduction

Colorectal cancer (CRC) is one of the most common cancers worldwide, with the highest mortality rate in both males and females [1]. Recent therapeutic approaches for advanced CRC still rely on surgery combined with adjuvant therapy such as chemotherapy or radiotherapy [2]. According to clinical data, 5% of CRC cases are associated with ulcerative colitis (UC) [3]. UC is a complex disorder that arises from a combination of genetic predisposition, defects in the epithelial barrier, dysregulated immune responses, environmental factors, and an imbalance between gut microbiota and mucosal immunity [4]. The immune response and inflammatory

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pathways associated with UC have been shown to involve the interplay of various cells and cytokines, resulting in tissue damage [4-7]. A positive correlation exists between the severity and duration of colitis and the likelihood of developing CRC [5]. A substantial body of clinical evidence indicates that individuals diagnosed with UC face an elevated likelihood of developing CRC [6].

Various risk factors have been identified as potential contributors to the development of CRC, including genetic predisposition, environmental influences, and the presence of gut microbiota [7]. Therefore, initial CRC screening is crucial for UC patients to enable early detection and intervention. Current treatments for CRC are often associated with severe side effects and a high recurrence rate, particularly in high-risk populations such as patients with UC [8]. This limitation highlights the need for effective and less harmful alternative or complementary strategies. Natural-based therapy from medicinal plants has gained attention as a potential agent for CRC prevention, one of which is *Beta vulgaris*. Commonly known as beetroot, *B. vulgaris* possesses a range of nutritional components including carbohydrates, fats, micronutrients, and various bioactive compounds such as betaine, polyphenols, carotenoids, flavonoids, saponins, and betalains [9]. These bioactive compounds have been reported to exhibit potent anti-inflammatory and antioxidant properties, which are beneficial to the digestive system [10,11]. Furthermore, betalain and phenolics isolated from beetroot have been found to promote the production of intestinal microbiota and probiotics [12]. Beetroot extract has also been shown to possess anticancer properties by promoting proapoptotic effects [13]. Given these benefits, it was hypothesized that beetroot extract may potentially prevent the progression of CRC. Therefore, the aim of this study was to identify the potential of beetroot to prevent the formation of CRC through differentially expressed gene (DEG) analysis and network pharmacology approaches.

Methods

Differentially expressed gene (DEG)

The present study involved the analysis of gene expression patterns in DEGs using the Gene Expression Omnibus (GEO) browser platform (https://www.ncbi.nlm.nih.gov/geo/). The GEO accession number GSE10972 was utilized for this analysis. A total of 24 samples in the dataset were utilized in this study, consisting of both CRC cells and normal mucosal cells. For the statistical analysis, the *p*-values were adjusted using the Benjamini and Hochberg method (False discovery rate, FDR) with a significance level set at 0.05. The quantification of upregulated and downregulated contigs, as determined by a *p*-value threshold of less than 0.05 and a log2FC value greater than or equal to 1, or less than or equal to -1.

Functional and enrichment analysis

Genes with significant differential expression from DEG analysis were annotated and analyzed for their biological roles. Gene ontology (GO) enrichment analysis was performed using the ShinyGo webserver (version 0.741, http://bioinformatics.sdstate.edu/go74/) with a *p*-value threshold of <0.05. GO terms were selected based on molecular function, biological process, and cellular components. Multiple testing correction was applied using the Benjamini and Hochberg method (FDR). Additionally, pathway enrichment analysis was conducted using the Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.genome.jp/kegg/) for *Homo sapiens* to identify associated biological pathways based on *p*-value threshold of <0.05. Significantly regulated genes involved in the pathway were subsequently analyzed for protein-protein interactions using the STRING database (https://string-db.org/), with a confidence threshold of 0.9. The interaction course was filtered based on the degree, betweenness, and closeness centrality network, with the focus on a highly connected network or cluster.

Beetroot extraction and bioactive compound screening process

The beetroot was purchased from local supermarket in Jakarta, Indonesia. The samples were then washed and diced into small pieces and air-dried at room temperature. The dried material was ground into fine powder using an electric grinder (Panasonic, New Delhi, India). The 500 mg beetroot powder was extracted using maceration technique with 96% ethanol as the solvent, with a 1:2 ratio for 24 hours. Gas chromatography-mass spectrometry (GC-MS) and liquid

chromatography-mass spectrometry (LC-MS) quadrupole time-of-flight (QTOF) analysis (18-16-2/MU/SMM-SIG (LC-MS/MS) QTOF) using the natural product library were performed to identify the bioactive compounds in the beetroot's ethanol extract. The chromatographic separation was conducted using C18 column (1.8 µm 2.1×100 mm, ACQUITY UPLC® HSS, Waters, USA). The mobile phase consisted of solvent A (formic acid/acetonitrile, 10:90 v/v) and solvent B (formic acid/deionized water, 10:90 v/v), which were delivered at a flow rate of 0.6 mL/min. The injection volume was 10 µL (initially filtered through a 0.2 µm syringe filter), and the chromatographic column was maintained at 40°C, while the autosampler was set at 15°C. The analysis was conducted in positive and negative electrospray ionization (ESI) modes.

Protein and ligand preparation

The cyclin-dependent kinase 4 (CDK4) crystal structure (PDB ID: 7SJ3) was acquired from the protein data bank (PDB) repository (https://www.rcsb.org/). The CDK4 protein is a protein that has undergone mutation, and its structure has been determined with a resolution of 2.51 Å [14]. The protein structure comprised two chains, namely chains A and B. Chain A, corresponded to CDK4, was employed for molecular docking. The indigenous ligands within the protein were spatially isolated, and the aqueous arrangement was eliminated. Hydrogen (with polar characteristics) was introduced into the system, followed by the application of an electric charge. Protein structures prepared for testing purposes were saved in a .pdbqt file format.

The ligand preparation process involves the utilization of Marvin Sketch software (Cheminformatics, Budapest, Hungary). Five ligands, namely quercetin, hesperidin, galangin, farrerol, and betanin, were employed as test compounds. The selection of those ligands was based on the Comparative Toxicogenomics Database (CTD) (https://ctdbase.org/) and confirmed by GC-MS analysis of the beetroot ethanol extract. The 3D structures of the five ligands were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in the structure-data file (SDF) format. The 3D conformation of each ligand was reconstructed using Marvin Sketch software and subsequently converted to PDB format. Subsequently, the ligand underwent torsion and was subsequently saved in a pdbqt format. The preparations of ligand and protein were performed on AutoDockTools 1.5.6 software (AutoDock, California, USA).

Molecular docking

The CDK4 protein (PDB ID: 7SJ3), possesses a native ligand that is situated within the receptor or active site of the CDK4 protein. The validation of the grid boxes was conducted using dimensions of 40×40×40 Å, 50×50×50 Å, and 60×60×60 Å (x,y,z), along with ten different running poses. We selected grid boxes containing a target protein-ligand complex with a reference root mean square deviation (RMSD) value of less than 2Å [15]. The docking process was conducted utilizing AutoDockTools 1.5.6 software (AutoDock, USA), employing a genetic algorithm and Lamarckian genetic algorithm (GA). The LigandScout application was employed to visually represent the 2D and 3D structure of the resulting protein-ligand complex. The preferable binding energy was between -4.00 and -10.00 kcal/mol [15].

MTT assay

HT-29 cell line was retrieved from the integrated laboratory, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia. A total of 10,000 HT-29 cells were dispensed into a 96-well plate and subjected to incubation for 24 hours. The ethanol extract derived from the maceration extraction technique was quantified at a mass of 10 mg and subsequently dissolved in 1 mL of 0.1% dimethyl sulfoxide (DMSO). The beetroot extract was dissolved and prepared into a series of concentrations, namely 400 µg/mL, 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, and 3.125 µg/mL, using Roswell Park Memorial Institute (RPMI). Complete medium containing 10% fetal bovine serum, 1% Penicillin-Streptomycin, and 1% amphotericin B. Doxorubicin was employed as the positive control of this study. The samples and doxorubicin were introduced into individual wells containing HT-29 cells and subsequently subjected to a 24-hour incubation in 37°C and 5% CO_2. The experiment involved the administration of a 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent, which was diluted ten times. This administration was conducted for four hours. Following this, DMSO was added as a stop solution. The optical density was then measured using microplate reader

(Thermo Scientific, 840-309400) at 590 nm. A line equation, y=ax+b, was derived from a curve that represents the relationship between the concentration of beetroot extract and the percentage of inhibition. A half-maximal inhibitory concentration (IC_{50}) was estimated from the standard curve. The concentration values of beetroot ethanol extract were log-transformed to meet statistical assumptions for dose-response analysis.

Results

Differentially expressed gene analysis

GEO Browser results with the accession number GSE10972 revealed that a total of 7,443 genes exhibited significant differential expression ($p<0.05$) between these two cell types [16]. A volcano diagram illustrating the distribution of the identified DEGs between colorectal tumor cells and normal mucosal cells is presented in **Figure 1**. Out of the 7,443 genes, a subset of 280 genes was chosen based on their $p<0.05$ and log2 value less than 2 fold change.

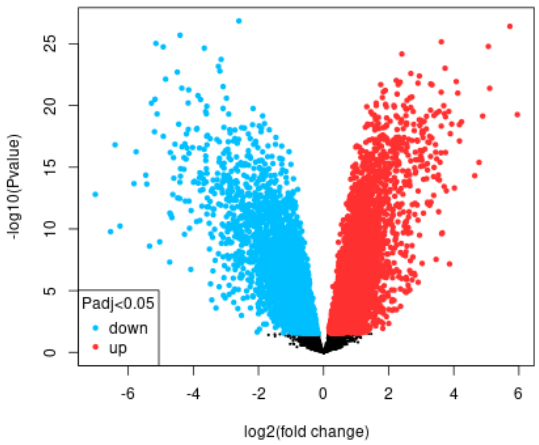


Figure 1. Volcano plot exhibiting significant differentially expressed gene (DEG) analysis of colorectal primary tumor cells and normal cells. Red and blue dots represent significant DEGs with $p<0.05$ and $1 \leq log2FC \leq -1$. Black dots represent non-significant DEGs.

Functional and enrichment analysis

Pathway enrichment analysis on the identified significant genes yielded a total of 18 pathways, where the results are presented in **Table 1**. This analysis revealed a significant enrichment of DEG in the cell cycle pathway. Genes that exhibit significant regulation within the cell cycle pathway are identified in **Table 1**. Specifically, 11 genes were identified, and these genes were associated with 126 pathways related to the cell cycle, as depicted in **Figure 2**. The network interaction was derived from K-means clustering with three clusters (**Figure 2**): cluster 1, represented in green, consisted of 17 genes; cluster 2, denoted in red, comprised nine genes; while cluster 3, indicated in blue, encompassed three genes. Specifically, the genes emphasized in red (**Figure 3**) hold significance in this regard. The interconnectivity of network relationships among genes and proteins associated with UC in cancerous pathways and the cell cycle results in interconnected interactions among all genes. The results of the GO analysis are presented in **Figure 4**.

Table 1. Annotation and pathway enrichment analysis

Sample	Pathway genes	Pathway	Genes
11	126	Cell cycle	*RBL1 CDC7 CDC25B CCNE1 MCM3 CDK4 TP53 SKP2 ANAPC1 MCM7 TFDP1*
12	294	MAPK signaling pathway	*ANGPT2 CDC25B MET VEGFA STMN1 PGF DU SP4 IL1B TP53 MAPK13 ELK4 EFNA4*
9	161	MicroRNAs in cancer	*CDC25B CCNE1 MET VEGFA STMN1 DNMT1 T P53 DDIT4 EFNA4*
6	77	Ribosome biogenesis in eukaryotes	*UTP18 RBM28 RPP40 UTP14A GNL3 RPP25*

Sample	Pathway genes	Pathway	Genes
6	92	Small cell lung cancer	*CCNE1 CDK4 TP53 SKP2 CKS1B LAMB3*
4	36	DNA replication	*RFC5 MCM3 MCM7 PRIM1*
4	41	Ferroptosis	*SLC11A2 STEAP3 SLC39A8 TP53*
8	222	Human T-cell leukemia virus 1 infection	*TCF3 CCNE1 CDK4 TP53 ANAPC1 ETS2 ELK4 MSX1*
3	31	Antifolate resistance	*IL1B SLC19A1 SHMT2*
10	354	PI3K-Akt signalling pathway	*ANGPT2 CCNE1 MET VEGFA PGF CDK4 TP53 DDIT4 LAMB3 EFNA4*
5	100	AGE-RAGE signalling pathway in diabetic complications	*PLCB4 VEGFA IL1B CDK4 MAPK13*
13	530	Pathways in cancer	*GSTO2 PLCB4 CCNE1 MET WNT2 VEGFA PGF BMP4 CDK4 TP53 SKP2 CKS1B LAMB3*
6	138	Fluid shear stress and atherosclerosis	*GSTO2 VEGFA BMP4 IL1B TP53 MAPK13*
7	210	Rap1 signaling pathway	*ANGPT2 PLCB4 MET VEGFA PGF MAPK13 E FNA4*
4	73	P53 signaling pathway	*CCNE1 STEAP3 CDK4 TP53*
6	167	Hepatocellular carcinoma	*GSTO2 MET WNT2 CDK4 ACTL6A TP53*
4	79	RNA degradation	*CNOT1 LSM8 HSPD1 PABPC3*
3	43	Type I diabetes mellitus	*ICA1 IL1B HSPD1*

The genes that play a role in the cell cycle in terms of biological processes, molecular function, and even cellular components are presented in **Figure 4**. The most essential molecular functions in this cell cycle process are DNA replication and double-stranded DNA binding. There are four genes involved in this molecular function; *RLB1, CDK4, CCNE1,* and *TP53*. Regarding the cellular component, the cell nucleus is where these molecular functions occur.

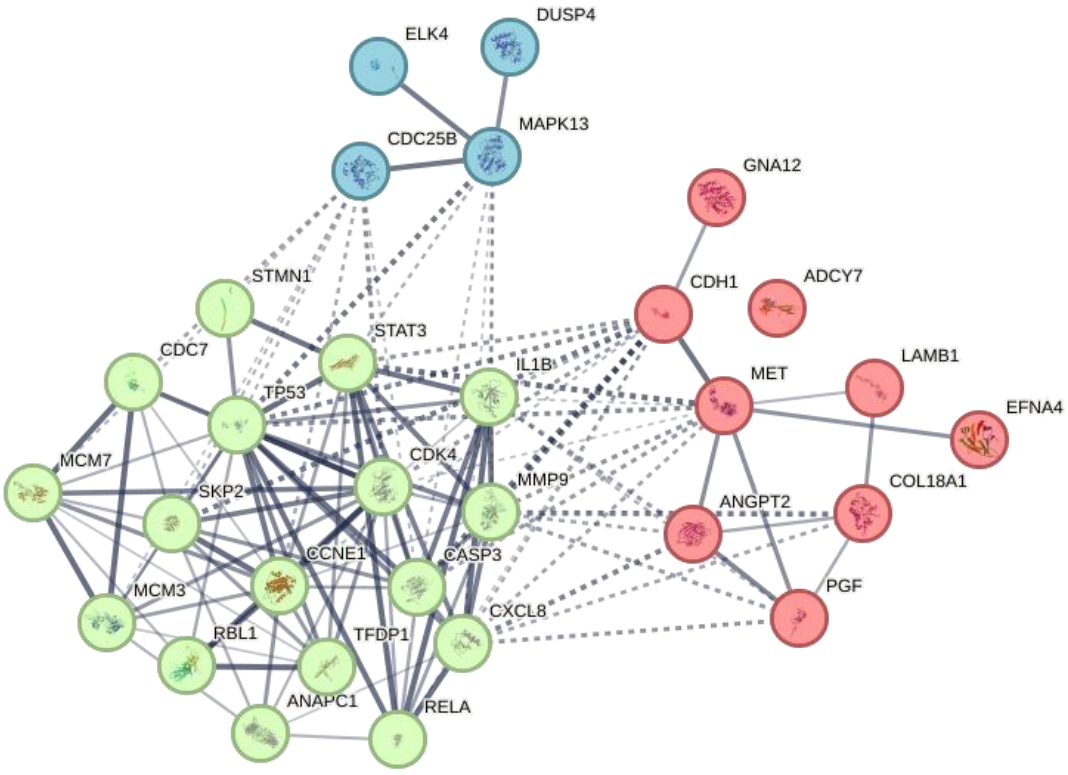


Figure 2. Protein network related to ulcerative colitis (UC) pathways in cancer and cell cycle with significant gene expression regulation. The thickness of the line indicates the more substantial the data supporting the protein/gene network interaction. Cluster 1: green nodes; cluster 2: red node; cluster 3: blue node.

A literature study was carried out regarding compounds in beets that could be active and were linked to the 11 genes using CTD to identify this mechanism. The compounds used refer to

the reported article [17]. The relationship between cell cycle-related genes and the biologically active compounds of beetroot is presented in **Table 2**.

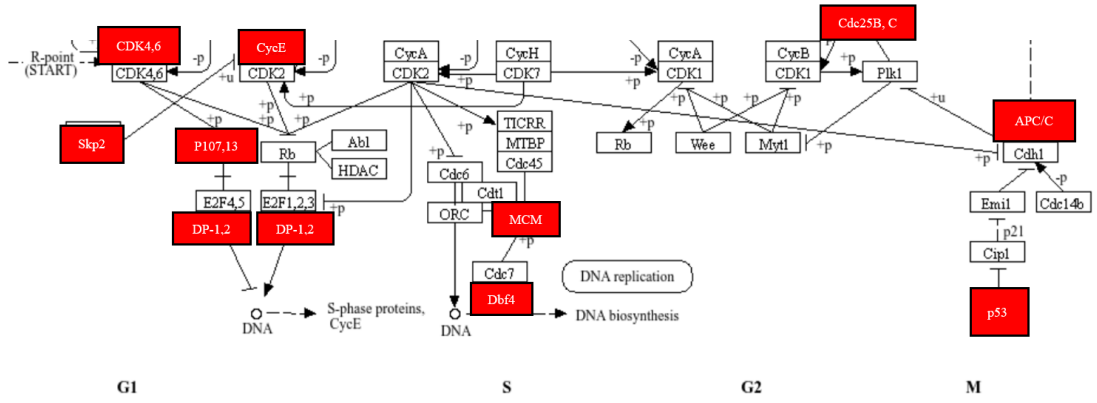
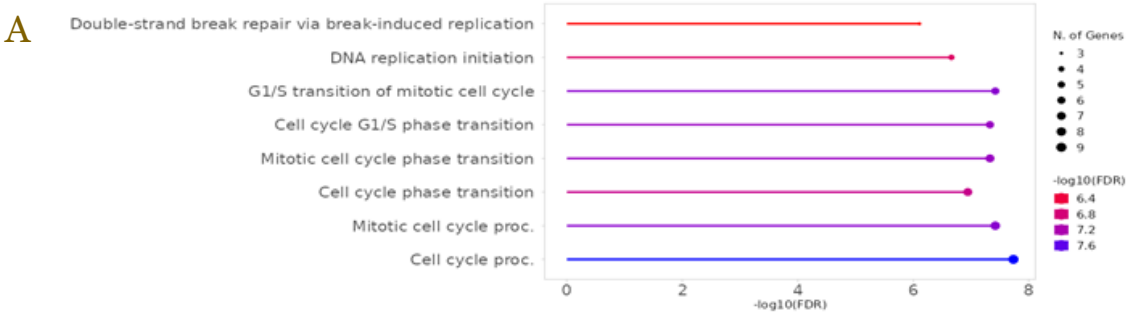
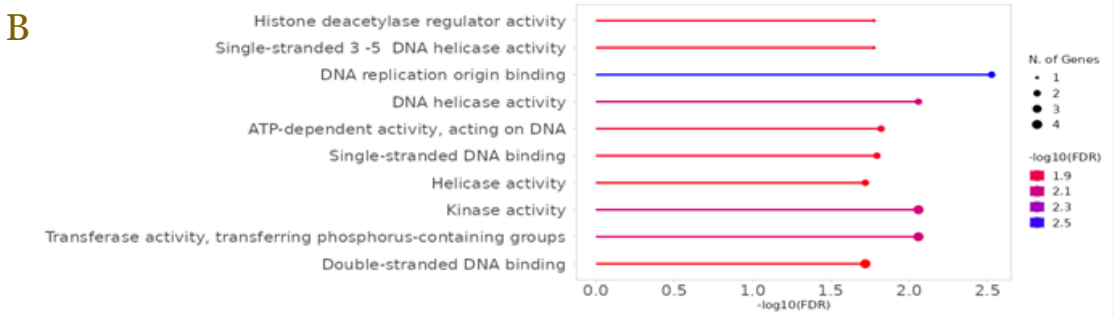


Figure 3. Mechanism of *Beta vulgaris* on the cell cycle (KEGG modification). Genes marked in red are genes that can influence regulation due to the addition of the active compound *B. vulgaris*.





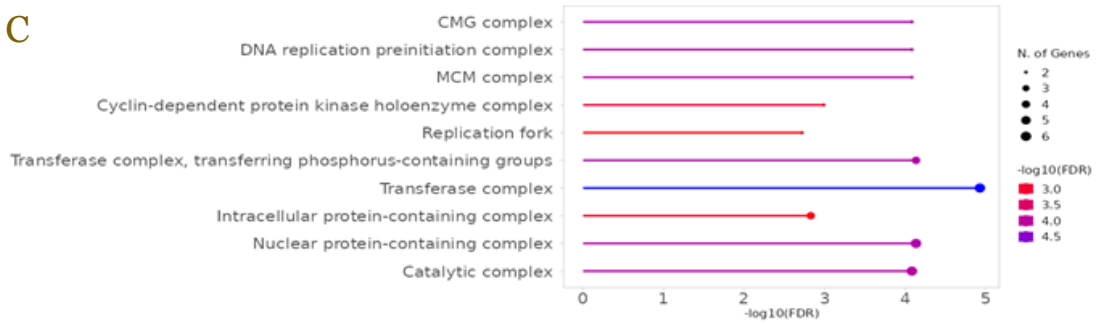


Figure 4. Gene ontology analysis related to cell cycle based on biological process (A), molecular function (B), and cellular components (C). The graphs were illustrated with blue to red gradient corresponding to the highest and lowest value, respectively.

Table 2. Relationship between active *Beta vulgaris* compounds that influence regulation of the cell cycle

Gene symbol	Compounds	Organism	Regulation
RLB1	Quercetin	*Homo sapiens*	Decreases expression
CDC7	Quercetin	*Homo sapiens*	Decreases expression
CCNE1	Quercetin	*Homo sapiens*	Increases expression
MCM3	Quercetin	*Homo sapiens*	Decreases expression
CDK4	Quercetin, galangin, hesperidin, farrerol, betanin	*Homo sapiens*	Decreases expression
TP53	Vanillin, formononetin, 3,3',4,5'-tetrahydroxystilbene, fisetin, betalains	*Homo sapiens*	Decreases expression
SKP2	Quercetin	*Homo sapiens*	Decreases expression
ANAPC1	Quercetin	*Homo sapiens*	Decreases expression
MCM7	Quercetin	*Homo sapiens*	Decreases expression
TFDP1	Quercetin	*Homo sapiens*	Decreases expression

Molecular docking analysis

A total of 40 compounds were identified from CTD database and GC-MS analysis. GC-MS result was presented in **Figure 5** and **Table 3**. Of which, quercetin, galangin, hesperidin, farrerol, and betanin exhibited high docking scores with CDK4 (-7.04, -7.13, -7.96, -6.28, and -8.11 kcal/mol, respectively). The selected compounds, based on their docking scores, are referred to as hit compounds and were analyzed for their interacting amino acids (**Table 4**). The docking results of five compounds, namely quercetin, galangin, hesperidin, farrerol, and the standard ligand, were examined. The 3D and 2D illustrations of the protein-ligand interactions are presented in **Figure 6**.

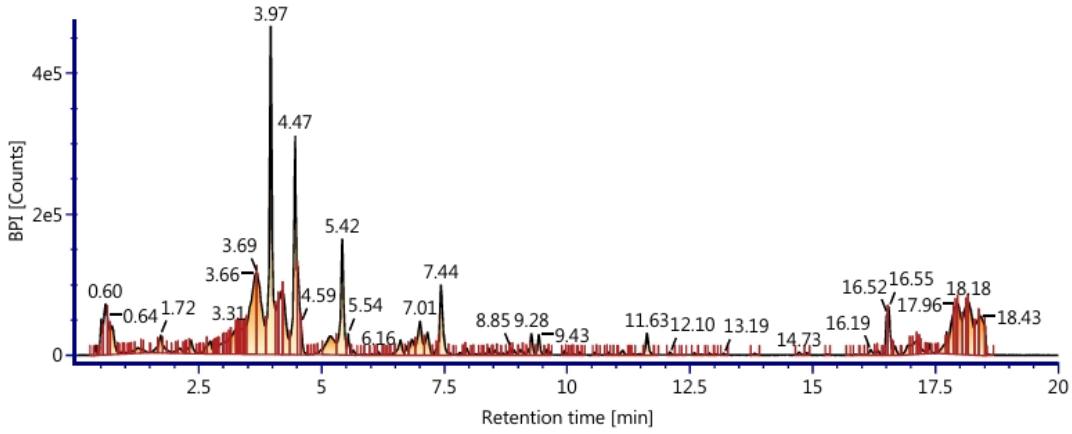


Figure 5. Chromatogram of GC-MS analysis of beetroot extract.

Table 3. GC-MS analysis of beetroot

No	ESI mode	Compound name	Result
1	(+)	5,7,8,3′,4′-Pentamethoxy flavone	Positive
2	(+)	Baicalein-7-O-β-D glucopyranoside	Positive
3	(+)	Chrysin	Positive
4	(+)	Genistein_1	Positive
5	(+)	Farrerol	Positive
6	(+)	Isoschaftoside	Positive
7	(+)	Kaempferol-3-O-β-D-glucopyranoside	Positive
8	(+)	Pinnatifinoside A	Positive
9	(+)	Hesperidin	Positive
10	(-)	Baicalein-7-O-β-D glucopyranoside	Positive
11	(-)	Cyanidin 3-glucoside	Positive
12	(-)	Eriodictyol-7-O-β-D-methyl-glucuronopyranoside	Positive
13	(-)	Eupatin	Positive
14	(-)	Kaempferol	Positive
15	(-)	Kushenol B	Positive
16	(-)	Limocitrin-3,7-O-β-D-glucopyranoside	Positive
17	(-)	Myricomplanoside	Positive
18	(-)	Nelumboroside B	Positive
19	(-)	Quercetin-3-O-α-L-rhamnose-7-O-β-D-glucoside	Positive

No	ESI mode	Compound name	Result
20	(-)	Galangin	Positive
21	(-)	Undulatoside A	Positive
22	(+)	2-Hydroxy-5-methyl-hypnone	Positive
23	(+)	Ehretioside B	Positive
24	(+)	Isocimicifugamide	Positive
25	(+)	Moupinamide	Positive
26	(+)	p-Hydroxyacetanilide	Positive
27	(-)	Ehretioside B	Positive
28	(-)	Octahydrocurcumin	Positive
29	(+)	Betanin	Positive
30	(+)	Cimidahuside G	Positive
31	(+)	Deoxyglabrolide	Positive
32	(+)	Ganoderic acid Y	Positive
33	(+)	Lucialdehyde A	Positive
34	(+)	Melianol	Positive
35	(+)	Mubenoside A	Positive
36	(+)	Phytolaccagenin	Positive
37	(-)	glucuronopyranosyl gypsogenin	Positive
38	(-)	Bidentatoside I	Positive
39	(-)	Phytolaccoside A	Positive
40	(-)	Poricoic acid C	Positive
41	(-)	Rutevin	Positive

ESI: electrospray ionization; ESI (-): ESI negative ion mode; ESI (+): ESI positive ion mode

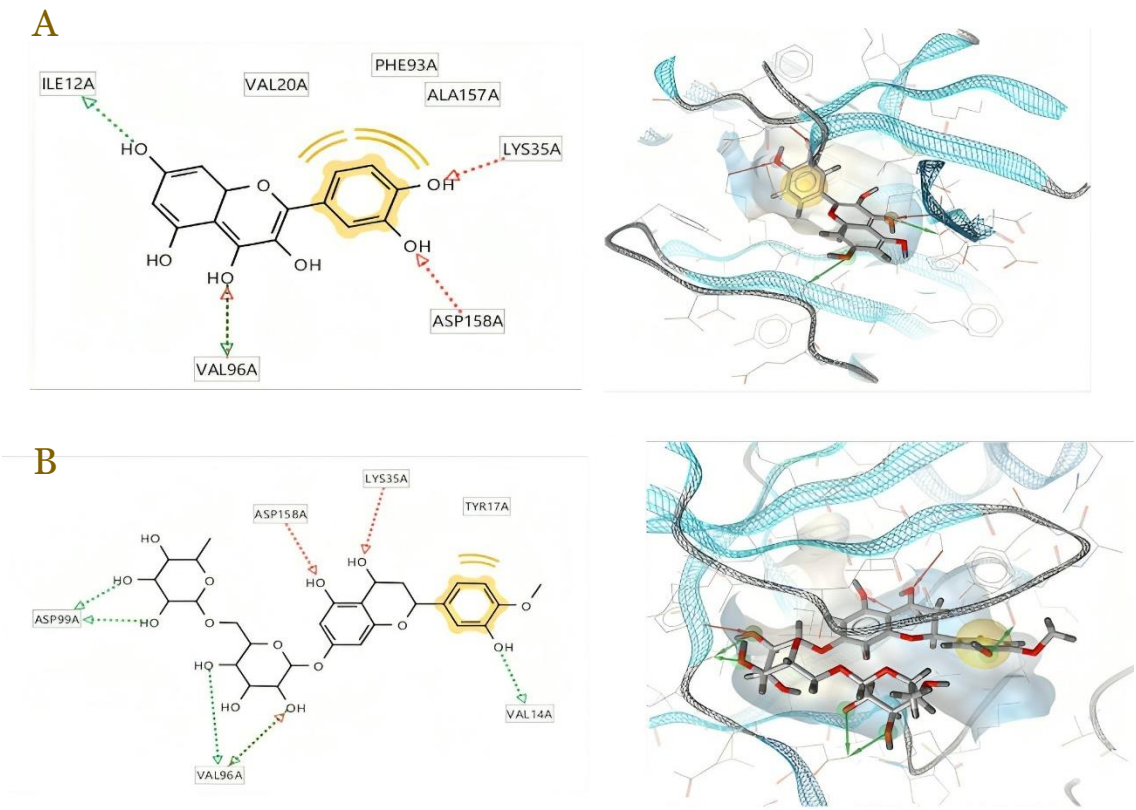


Figure 6. Two- and three-dimensional structures for the interactions of CDK4 with quercetin (A) and betanin (B).

Cytotoxicity analysis

The linear equation for beetroot extract yielded an R^2 value of 0.9912, whereas an R^2 value of 0.9878 was obtained for doxorubicin. There is a positive correlation between the concentration of beetroot extract and the rate of cell death. Beetroot exhibited IC_{50} of 39.03±1.4 µg/mL against the cancer cell line, presented as mean ± standard deviation (SD) from linear regression analysis based on dose-response curve (**Figure 7**).

Table 4. Molecular docking results of beetroot compounds with CDK4

Compounds	Free energy binding (ΔG) (Kcal/mol)	Ki (μM)	Amino acid interactions			
			H	HBA	HBD	Hydrophobic
Quercetin	-7.04	6.93	Asp158	Val20, Phe93, Ala157, Lys35	ILE12, Val96	Val20A, Phe93A, Ala157A
Hesperidin	-7.96	1.47	Asp99, Val96	Asp158, Lys35, Tyr17	Asp99	Tyr17
Farrerol	-6.28	24.92	Glu14, Glu94	Lys35	Ile12, Val20, Leu147, Ala157, Phe93, Val72, Ala33	Phe93, Val72, Ala33
Galangin	-7.13	5.99	Val96, Asp158	Ala157, Lys35, Val20	Glu94	Lys35, Val20
Betanin	-8.11	1.14	Lys35, Lys22	Val96	Asp158, Asp99	TYR17A

H: hydrogen bond; HBA: hydrogen bond acceptor; HBD: hydrogen bond donor

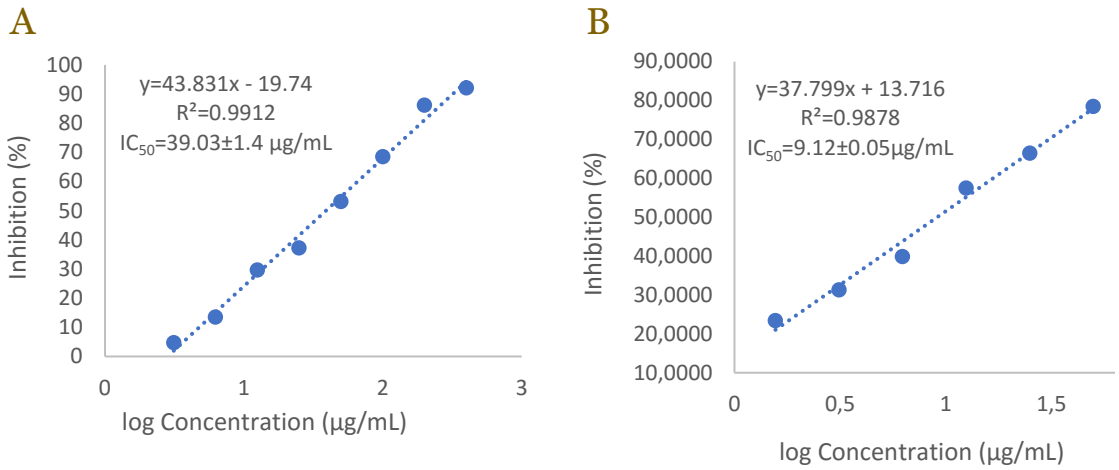


Figure 7. Linear regression curve of log concentration against % inhibitions of beetroot extract (A) and doxorubicin (B).

Discussion

Results in the present study revealed 10 out of the 11 genes associated with cell cycle regulation exhibited altered expression levels upon exposure to compounds contained in beetroot extract. Notably, quercetin was involved in the regulation of all genes, except the *TP53*. Typically, beetroot compounds downregulate genes associated with the cell cycle, potentially counteracting mutations that lead to uncontrolled cell proliferation. Among the five selected compounds based on the docking scores, betanin exhibited superior performance with CDK4 as the protein target. The ligand formed a complex with the target, resulting in the formation of a hydrogen bond involving hydrogen bond donor (HBD), hydrogen bond acceptor (HBA), and hydro-phobic interactions with specific amino acid residues. In addition to exhibiting a superior docking score, betanin demonstrates a more favorable predicted Ki (constant inhibition) value compared to other ligands. The Ki, expressed in micromolar units, is relatively minor, indicating that betanin exerts a significant effect despite its low Ki value. This result possessed the potential in modulating cell cycle by inhibiting CDK4 protein. These findings suggest that beetroot extract has the potential to modulate gene expression within the CRC cell cycle.

This study showed significant enrichment of DEG in the cell cycle pathway, including CDKs. CDK genes play a crucial role in the regulation of cell cycle dynamics by controlling the activation of retinoblastoma 1 (RB1) protein [18]. The RB protein is phosphorylated by CDK4 and CDK6, and subsequently by cyclin E– and A–dependent CDK2. The overall activity of cyclin-dependent kinases (CDKs) exhibits an upward trend throughout the cell cycle [19]. RB1 phosphorylated during the transition from the S phase to the M phase [20]. At the same time, the other protein, called cell division cycle 25B (Cdc25B) exhibits an increase starting from the S-phase and reaches

its maximum concentration during the G2/M phase[21,22]. The degradation of the Cdc25B protein is promptly facilitated by the proteasome pathway after the completion of the M phase in the cell cycle [23-26]. The overexpression of CDC25B was also identical to oncogenic characteristics [27]. On the other hand, the G1 phase of the cell cycle is initiated by E2F that regulated by mini chromosome matrix (*MCM*) protein genes, specifically *MCM2-7* [27,28]. It has been observed that an increase in the expression of *MCM* genes is associated with the development of cancer [29].

The orderly progression of the cell cycle is crucial for maintaining cellular homeostasis. This process relies on the interaction between cyclins, CDKs, and CDK inhibitors (CDKIs) [29]. Quercetin induced cell cycle arrest through its modulation of various target proteins, including p53, p21, p27, cyclin B, cyclin D, and CDK. Quercetin could decrease the expression of cyclin B1 and CDK-1, both of which play a crucial role in facilitating the smooth transition of cells from the G2 phase to the M phase of the cell cycle [30]. Similarly, both quercetin and betanin have been reported to induce G1/S arrest by upregulating p21 and phosphorylating the retinoblastoma protein (pRb), ultimately inhibiting E2F1 activity and impeding cell cycle progression [31]. Notably, molecular docking results have revealed high binding affinity between betanin and CDK4, further supporting its potential role in disrupting cell cycle regulation at the G1 phase. This strong interaction suggests that betanin may act as an effective inhibitor of CDK4, reinforcing its ability to induce cell cycle arrest and contributing to its anticancer properties.

The cytotoxic effects of beetroot extract on the HT29 Cell line have been demonstrated by in vitro investigations. Additionally, notable synergistic effect was observed when cancer cells were simultaneously exposed to beetroot extract and the chemotherapeutic agent doxorubicin [32]. Various studies have demonstrated that betanin, the primary component found in red beetroot, promotes anticancer and antiproliferative properties by stimulating antioxidant defense mechanisms and scavenging lipoperoxyl free radicals [33,34]. Furthermore, betanin may have a significant impact on the prevention of liver, lung, and kidney injuries and cancer through the induction of detoxifying and antioxidant enzyme expression, as well as the reduction of xenobiotic-induced oxidative stress [35,36]. The efficacy of red beetroot extract (*B. vulgaris* L.), an FDA-approved red food color (E162), has been demonstrated in reducing the occurrence of experimental tumors in various organs [37]. Consequently, it holds significant potential as a natural product for the prevention and treatment of human cancers. Moreover, beetroot exhibits potential chemo-preventive properties, as evidenced by its ability to delay the onset of tumors, increase tumor latency, decrease tumor multiplicity, release reactive oxygen species (ROS), and further reduce splenomegaly [38,39].

Despite the promising findings, this study has several limitations, while the study highlights the differential expression of cell cycle-related genes following exposure to beetroot extract, the underlying mechanisms driving these changes remain unclear. Additional transcriptomic or proteomic analyses would be beneficial to elucidate how beetroot compounds regulate gene expression comprehensively. The other beetroot-derived compounds may also contribute to the observed effects, and their potential roles warrant further investigation. Addressing these limitations in future research will provide a more comprehensive understanding of the anticancer potential of beetroot-derived compounds.

Conclusion

Beetroot extract has the potential to prevent CRC formation by significantly downregulating gene expression in the cell cycle pathway. Molecular docking analysis revealed a strong binding affinity between betanin and CDK4, suggesting its role in inducing G1/S cell cycle arrest. Additionally, the in vitro studies demonstrated the cytotoxicity of beetroot extract on CRC cells (HT29) cell line with IC_{50} of 39.03±1.4 µg/mL.

Ethics approval

Not required.

Acknowledgments

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Competing interests

All the authors declare that there are no conflicts of interest in this study.

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Underlying data

All of the data of this study are available in this manuscript.

Declaration of artificial intelligence use

This study used artificial intelligence (AI) tools and methodologies in the following capacities of which AI-assisted techniques (DEG, ShinyGo, STRING, KEGG) were applied for data preprocessing, analysis, and modelling. AI-based language model was employed for language refinement (improving grammar, sentence structure, and readability of the manuscript). We confirm that all AI-assisted processes were critically reviewed by the authors to ensure the integrity and reliability of the results. The final decisions and interpretations presented in this article were solely made by the authors.

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