© 2022 Journal of Pharmacy & Pharmacognosy Research, 10 (4), 595-604, 2022
ISSN 0719-4250
https://jppres.com

DOI: https://doi.org/10.56499/jppres21.1321.10.4.595

Original Article

Effect of 1,4-naphthoquinone from *Sisyrinchium palmifolium* L. extract on *in vivo* Ki-67 expression and *in silico* CDK1, CDK2, CDK4 on colitis-associated colon cancer

[Efecto de la 1,4-naftoquinona del extracto de *Sisyrinchium palmifolium* L. sobre la expresión de Ki-67 *in vivo y* CDK1, CDK2, CDK4 *in silico* en el cáncer de colon asociado a colitis]

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Abstract

Context: Medicinal plants can be used as an option for the prevention and reduction of cancer cell resistance and its side effects. *Sisyrinchium palmifolium* L. is thought to have anti-cancer activity with a compound content of 1,4-naphthoquinone.

Aims: To determine the effect of *S. palmifolium* extract (SPE) with the main compound 1,4-naphthoquinone on Ki-67 expression by *in vivo*, and CDK1, CDK2, and CDK4 activity by *in silico* in colonic epithelial cells of BALB/ c mice induced by azoxymethane (AOM) dextran sodium sulfate (DSS).

Methods: Dayak onion (*S. palmifolium*) was extracted using 96% ethanol as a solvent. The *S. palmifolium* extract was then made into tablet form by the wet granulation method. Mice that had been induced with AOM-DSS were given *S. palmifolium* extract therapy. Twenty samples were used, which were divided into five groups. Mice colon tissue was assessed using Ki-67 immunohistochemistry. This study also used the *in silico* method to see the effect of 1,4-naphthoquinone compounds from *S. palmifolium* extract on the expression of CDK1, CDK2 and CDK4 with PDB codes 6GU6, 6GUC, and 1GIH.

Results: Ki-67 expression values were 26 ± 6.51 cells at low dosages, 15 ± 1.73 cells at moderate doses, and 11 ± 1.04 cells at high doses. Between the test groups, there was a statistical differences ($p<0.05$) with the Post Hoc Mann-Whitney test. At the 6GUC receptor, the mean rerank score of the 1,4-naphtoquinone molecule, which was closest to the native ligand, was -54.6572 ± 2.2722 and -90.5455 ± 1.6524 kcal/mole. The steric bond on the amino acid lys 33 (A), which exclusively occurs at the 6GUC receptor, was the only commonality of contact.

Conclusions: 1,4-Naphthoquinone from *Sisyrinchium palmifolium* L. extract could decrease Ki-67 expression by *in vivo*, which cloud induce a decrease in epithelial cells proliferation in colon cancer, but has no potential as an inhibitor activity of CDK1, CDK2, and CDK4 by *in silico*.

Keywords: Dayak onion; immunohistochemistry; 1,4-naphthoquinone.

Resumen

Contexto: Las plantas medicinales pueden usarse como una opción para la prevención y reducción de la resistencia de las células cancerosas y sus efectos secundarios. Se cree que *Sisyrinchium palmifolium* L. tiene actividad anticancerígena con un contenido compuesto de 1,4-naftoquinona.

Objetivos: Determinar el efecto del extracto de *S. palmifolium* (SPE) con el compuesto principal 1,4-naftoquinona sobre la expresión de Ki-67 *in vivo* y la actividad de CDK1, CDK2 y CDK4 *in silico* en células epiteliales colónicas de ratones BALB/c inducida por azoximetano (AOM) dextrano sulfato de sodio (DSS).

Métodos: La cebolla de Dayak (*S. palmifolium*) se extrajo usando etanol al 96% como solvente. Luego, el extracto de *S. palmifolium* se transformó en forma de tableta mediante el método de granulación en húmedo. Los ratones que habían sido inducidos con AOM DSS recibieron terapia con extracto de *S. palmifolium*. Se utilizaron veinte muestras, las cuales se dividieron en cinco grupos. El tejido de colon de ratones se evaluó usando inmunohistoquímica Ki-67. Este estudio también usó el método *in silico* para ver el efecto de los compuestos de 1,4-naftoquinona del extracto de *S. palmifolium* sobre la expresión de CDK1, CDK2 y CDK4 con los códigos PDB 6GU6, 6GUC y 1GIH.

Resultados: Los valores de expresión de Ki-67 fueron 26 ± 6,51 células a dosis bajas, 15 ± 1,73 células a dosis moderadas y 11 ± 1,04 células a dosis altas. Entre los grupos de prueba, hubo diferencias estadísticas ($p<0,05$) con la prueba Post Hoc Mann-Whitney. En el receptor 6GUC, la puntuación de reclasificación media de la molécula de 1,4-naftoquinona, que era la más cercana al ligando nativo, fue -54,6572 ± 2,2722 y -90,5455 ± 1,6524 kcal/mol. El enlace estérico en el aminoácido lys 33 (A), que ocurre exclusivamente en el receptor 6GUC, fue el único elemento común del contacto.

Conclusiones: La 1,4-naftoquinona del extracto de *Sisyrinchium palmifolium* L. podría disminuir la expresión de Ki-67 *in vivo*, lo que induce una disminución en la proliferación de células epiteliales en el cáncer de colon, pero no tiene potencial como inhibidor de la actividad de CDK1, CDK2 y CDK4 *in silico*.

Palabras Clave: cebolla Dayak; inmunohistoquímica; 1,4-naftoquinona.

ARTICLE INFO
Received: November 27, 2021.
Received in revised form: January 29, 2022.
Accepted: February 3, 2022.
Available Online: March 31, 2022.

INTRODUCTION

Cancer is caused by abnormal cells in their growth and development, characterized by uncontrolled cell proliferation, invasiveness, and the ability to spread to surrounding body parts or other organs (ACS, 2017). Colon cancer is the world's second leading cause of cancer, accounting for 881 000 deaths in 2018 from 1.8 million cases (IARC, 2018). The factors that cause colon cancer are not known for certain. The occurrence of colon inflammation (colitis) can increase the risk of colon cancer. Chronic digestive tract inflammation characterized by irritation or injury is called inflammatory bowel disease (IBD) (Hwang et al., 2017). Colon cancer that begins with IBD is called colitis-associated colon cancer (CACC) (Grivennikov, 2013). Exposure to chemicals, radiation, and abnormal metabolism in the body can cause DNA damage. This will trigger the activation of the K-Ras pathway through the PI3K pathway, β-catenin, and mutations in tumor suppressor genes such as p53 mutations. Furthermore, it can trigger a decrease in cell apoptosis and increase the cell cycle in cancer (Marmol et al., 2017).

In colon cancer, there is overexpression of cyclin-dependent kinase 1 (CDK1), CDK2, and CDK4, which work in the cell cycle to have an important role in the proliferation of these cancer cells (Mikhail et al., 2015). An increase in the cell cycle in colon cancer indicates an increase in the proliferation and development of cancer cells. Early examination is needed to monitor and assess the patient's prognosis level so that cancer prevention efforts can be carried out to a higher stage. An excellent marker for determining the fraction of cell growth and development is the Ki-67 protein. The expression of Ki-67 is strongly associated with the proliferation and growth of cancer cells. The more malignant the cancer cells, the higher the expression of Ki-67, which can be used to detect recurrence. The peak of Ki-67 expression occurs in the mitotic phase (M) (Tadbir et al., 2012; Afiati, 2013).

Natural or herbal treatment is an alternative that has the potential to inhibit the growth and development of cancer cells, such as the Dayak onion plant (*Sisyrinchium palmifolium* L., family *Iridaceae*) (Syarif, 2014). Based on research, it shows that the ethanol extract of *S. palmifolium* can inhibit the growth of HeLa cervical cancer cells (Muti'ah et al., 2018). Another study showed that the administration of ethanol extract of *S. palmifolium* could trigger the apoptotic activity of cancer cells both intrinsically and extrinsically (Muti'ah et al., 2020). This is supported by previous studies, which showed that *S. palmifolium* could function as colonic anti-cancer agents (Li et al., 2008).

The content of the compound 1,4-naphthoquinone is a major compound tested for its existence in the extract of *S. palmifolium*. In the research, Annisa et al. (2020) stated that the compound content of 1,4-naphthoquinone in the *S. palmifolium* extract had the same retention and intensity as the pure compound. This compound has bioactivity as an anti-cancer and antioxidant (Kuntorini et al., 2016; Zhang et al., 2018). This study aimed to determine the effect of *S. palmifolium* extract (SPE) with the main compound 1,4-naphthoquinone on Ki-67 expression by *in vivo*, and CDK1, CDK2, and CDK4 activity by *in silico* in colonic epithelial cells of BALB/c mice induced by azoxymethane (AOM) dextran sodium sulfate (DSS).

MATERIAL AND METHODS

Plant material and extraction

Dayak onion (*Sisyrinchium palmifolium* L.) samples were obtained from Central Kalimantan (-2°00'0.00" S 113°30'0.00" E), Indonesia and were identified and approved by the UPTD Materia Medika Batu, Malang in letter number 074/348/102.7/2017. The extraction process was carried out using the ultrasonic-assisted extraction (UAE) method using 96% ethanol as a solvent. The extract was then rotated in a rotary evaporator and dried in an oven at 40°C. The outcome was Dayak onion blackish-red dry extract. Then, the extract was made into tablets by the wet granulation method, with a composition according to Table 1. Each tablet contained 50 mg of *S. palmifolium* extract (SPE).

Table 1. Composition of *S. palmifolium* tablets.

Materials	Concentration (%)
S. palmifolium extract	10
Maize starch	5
Aerosil	1
Polyvinyl pyrrolidone	5
Magnesium stearate	1
Talk (natural hydrated magnesium silicate)	2
Avicel	38
Lactose	38

Animals

Female BALB/c mice weighing 20-25 g and 8-10 weeks were exposed under a relative humidity of 50-55% and a predetermined light-dark cycle (12:12 h). During the trial, mice were fed with conventional pellets and were given normal drinking water *ad*

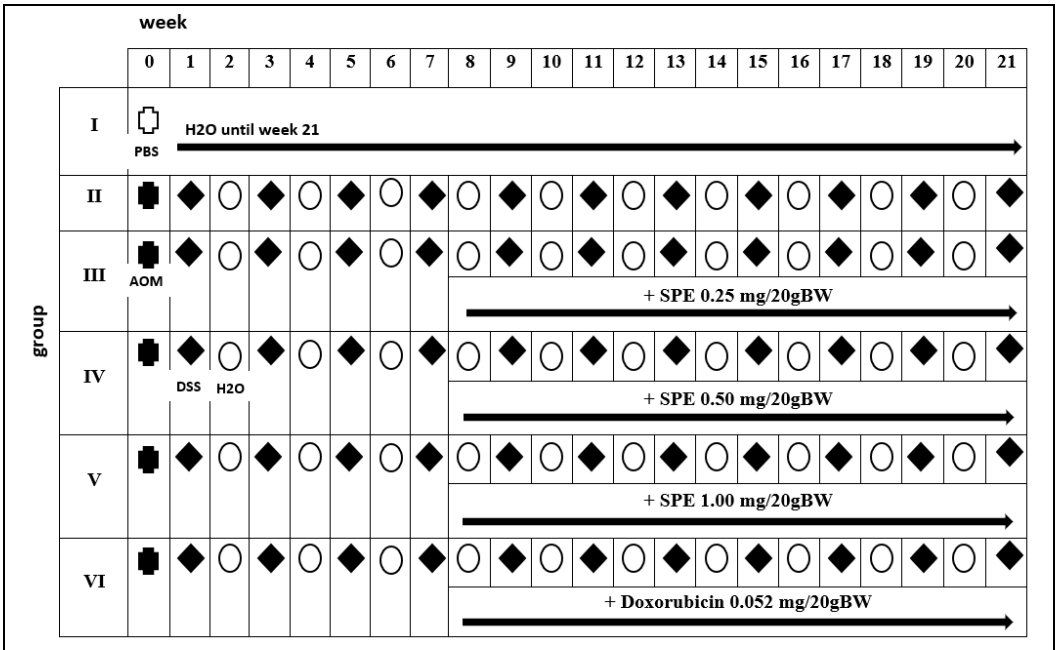

Figure 1. The experiment design of mice was induced colitis-associated colon cancer and SPE administration.

Firstly, the mouse was induced by azoxymethane (AOM) and then given drinking water containing 5 % dextran sodium sulfate (DSS) in a cycling protocol for seven days and followed by seven days of regular water alternately each week. DSS at weeks (1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21) and regular water at weeks (2, 4, 6, 8, 10, 12, 14, 16, 18 and 20). For the experiment, mice were divided into 6 groups; group I received water (negative control); group II was given DSS only (positive control); III-V groups were treated SPE (0.25 mg/20 g BW, 0.50 mg/20g BW and 1.00 mg/20g BW), respectively; group VI was given doxorubicin 0.052 mg/20 g BW (reference drug). Administrations of SPE and doxorubicin were started from the 8th week until the 21st week. Mice in all groups were sacrificed at the end of the 21st week. ⬡ = PBS (phosphate-buffered saline), ⬢ = AOM, ◆ = DSS, ○ = water (H_2O).

libitum. The experiment was performed based on the Guiding Principles for the Care and Use of Animals for Scientific Purposes of the Institutional Committee on the Care and Use of Animals (IACUC). The experiment had met the current regulations and ethical approval of the Health Research Ethics Committee of the Health Polytechnic of Malang, Indonesia (No.027/KEPK-POLKESMA/2019). The experiments took place during the light period with six treatment groups, each of which consisted of four mice tested in a randomized order.

AOM and DSS-induced colon cancer model

On day 1, mice were given a single intraperitoneal injection of 10 mg/kg azoxymethane (AOM; Sigma-Aldrich, UK) or vehicle (phosphate buffer saline, PBS). At one-week post-injection, colitis was induced by providing drinking water containing 5% DSS (ICN Biomedical Inc, CA, USA) for a week. DSS was administered in a cycling protocol followed by one week of regular water treatment. Colon cancer was induced through cyclical DSS treatment of 5% DSS administration for one week followed by seven days of regular water. The oral administration of SPE started at week 8 until 21 (Pattanayak et al., 2014). Mice were randomly divided into six groups (Fig. 1). Group I received water (vehicle) as a negative control group. Group II was given 5% DSS only; it was a positive control. Group III-V received SPE at 0.25, 0.5, or 1 mg/20 g body weight, and group VI received doxorubicin 0.052 mg/20 g body weight (BW), a reference drug. Colon tissue was removed and cleaned, then subjected to immunohistochemistry.

Ki-67 immunohistochemistry

Cell proliferation patterns in colon tissue were assessed using Ki-67 immunohistochemistry. For example, the paraffin-embedded portions of the colon tissue were deparaffinized and hydrated. A Tris-HCl buffer (0.05 M, pH 7.6) was used to prepare the solution for rinsing the slides between the various steps. Incubation was carried out in a humidified chamber, treated for 40 minutes at room temperature with 2% bovine serum albumin, and incubated overnight at 4°C with the mouse monoclonal primary antibody Ki-67 (6G6). Monoclonal antibody type bsm-52455R (Bioss, USA) 1:200 dilution for 1 hour at room temperature was used. The expression of Ki-67 was observed microscopically using a light microscope and obtained a brown color because the anti-Ki-67 antibody reaction was visualized with the DAB (3,3′-diaminobenzidine) chromogen (Sigma Aldrich, UK).

Observation of Ki-67 expression

A light microscope (Olympus) was used in each group to observe 10 visual fields at 400 times magnification, which showed that the unit used was cells in one field of view. The Ki-67 expression was calculated using the Fiji Program (ImageJ) in the nucleus of colon epithelial cells that showed a brown color.

Protein and ligand preparation

The three-dimensional structure of the compound 1,4-naphthoquinone was downloaded from the PubChem website and redrawn using the Chemdraw Ultra Version 12.0 application. Protein Data Bank (PDB) showed codes of 6GU6, 6GUC, and 1GIH for CDK1, CDK2, and CDK4. The 1,4-naphthoquinone compound was drawn using the Chem Bio Draw Ultra program version 12 on the SwissADME online application based on the Lipinski Rule of five (Ro5) as the parameter. The 3D compound 1,4-naphthoquinone was retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov), which was then minimized in the Avogadro program. The protein receptors of CDK1, CDK2 and CDK4 with codes of 6GU6, 6GUC, and 1GIH were retrieved from the Protein Data Bank (PDB) website (https://www.rcsb.org). The receptors underwent were validated three times for each receptor and were selected based on the RMSD value of less than 2 Å.

Molecular docking

The Molegro Virtual Docker 6.0 software was used to remove molecules from the receptor. After that, the cavity was detected. Only cavities with original ligand were selected. The 3D structure of the compound was placed into the selected cavity. The strength of the ligand was measured by the receptors, and results were then presented in rerank score. At this stage, amino acids were formed.

Toxicity test with Protox online tool application and pkCSM

The SMILES code of 1,4-naphthoquinone was used in the pkCSM online tool (http://biosig.unimelb.edu.au/pkcsm/prediction), which aims to predict Ames toxicity, hepatotoxicity, and skin sensitization. Meanwhile, compound toxicity (LD_{50}) was predicted using the online Protox tool (https://tox.charite.de/protox_II/).

Statistical analysis

The mean ± standard deviation (SD) was used in data presentation. The standard deviation test was performed using Microsoft Excel 2016 (Microsoft Corporation, Redmond, USA). Furthermore, statistical analysis was performed using the Statistical Program Service Solution (SPSS) 23, while the data were analyzed in the Kruskall-Wallis test ($p<0.05$). The process was then followed by the Mann-Whitney Post Hoc test performed to see groups with significant differences.

RESULTS

SPE decreases the expression of Ki-67 BALB/c mice in the CAC model

In this study, the expression of Ki-67 was observed using anti-Ki-67 staining, which was carried out by immunohistochemistry and observed using a microscope with a magnification of 400×. The Ki-67 expression indicates the proliferation of epithelial cells. The results showed a decrease in Ki-67 expression in BALB/c mice model CAC due to SPE treatment, results in each group were as follows: Negative control: 6.4 ± 1.48 cells, Positive control: 36.1 ± 12.57 cells, SPE 0.25 mg/20 g BW: 26.1 ± 6.51 cells, SPE 0.50 mg/20 g BW: 15.2 ± 1.74 cells, SPE 1.00 mg/20 g BW: 11.1 ± 1.09 cells, Doxorubicin 0.052 mg/20 g BW: 8.1 ± 0.56 cells. (Fig. 2B). The higher the dose of SPE given, the lower the Ki-67 expression. Statistical analysis showed that the negative control group had a significant difference ($p<0.05$) compared to the SPE group at a dose of 1.00 mg/20 g BW. The positive control group had a significant difference compared to the SPE group with doses of 0.50 mg/20 g BW and 1.00 mg/20 g BW (Fig. 2B).

Based on Fig. 2A (a-f), that amount of Ki-67 expression marked with brown color in epithelial cells shows less Ki-67 at higher SPE doses, a decrease in the proliferative activity of colonic epithelial cells that occurred in the SPE therapy group. In this study, SPE can reduce the proliferation of epithelial cells that suppress carcinogenesis.

Docking score

In Fig. 3A, the rerank score that was carried out three times between native ligands and 6GU6 receptors was -46.28, -49.56, and -49.09, so the average value was -48.31, while the rerank score between 1,4-naphthoquinone compounds and 6GU6 receptors was -112.36, -115.56, and -113.24, so the mean value was -114.05. The difference in the mean rerank score, which was quite far between the native ligand and 1,4-naphthoquinone, namely -48.31 and -114.05, indicates that the difference in activity was quite far. Therefore, 1,4-naphthoquinone did not effectively bind to the 6GU6 receptor, a CDK1 receptor protein. This means 1,4-naphthoquinone has little effect on inhibiting CDK1 activity in the cell cycle.

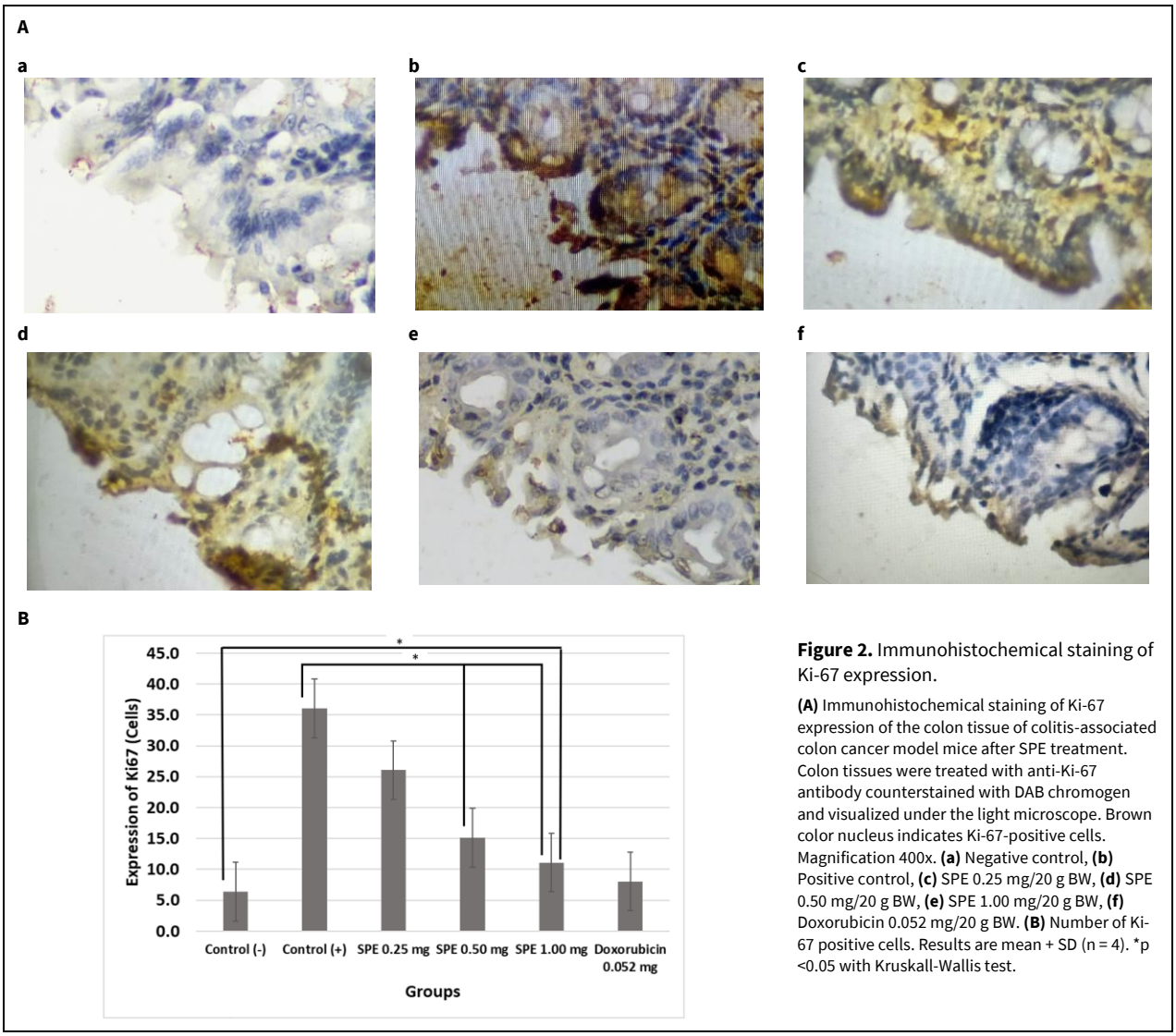

Figure 2. Immunohistochemical staining of Ki-67 expression.

(A) Immunohistochemical staining of Ki-67 expression of the colon tissue of colitis-associated colon cancer model mice after SPE treatment. Colon tissues were treated with anti-Ki-67 antibody counterstained with DAB chromogen and visualized under the light microscope. Brown color nucleus indicates Ki-67-positive cells. Magnification 400x. **(a)** Negative control, **(b)** Positive control, **(c)** SPE 0.25 mg/20 g BW, **(d)** SPE 0.50 mg/20 g BW, **(e)** SPE 1.00 mg/20 g BW, **(f)** Doxorubicin 0.052 mg/20 g BW. **(B)** Number of Ki-67 positive cells. Results are mean + SD (n = 4). *p <0.05 with Kruskall-Wallis test.

In Fig. 3B, the rerank score that was carried out 3 times between native ligand A and 6GUC receptors was -55.97, -55.97 and -52.03, so the average value was -54.66, while the rerank score between 1,4-naphthoquinone and 6GU6 receptors was -88.64, -91.45 and-91.55, so the average value was -90.55. In Fig. 3C, the rerank score between native ligand B and 6GUC receptors was -50.67, -50.60, and -50.61, so the mean value was -50.61, while the rerank score between 1,4-naphthoquinone and 6GU6 receptors was -90.31, -90.38, and -90.31, so the average value was -90.33. The significant difference in the average rerank score between native ligands A and B with 1,4-naphthoquinone, which were -54.66 with -90.55 and -51.61 with -90.33, indicates that the difference in activity was quite large. Therefore, 1,4-naphthoquinone did not effectively bind to 6GUC receptors, which are CDK2 receptor proteins. This means 1,4-naphthoquinone could have little effect on inhibiting CDK2 activity in the cell cycle.

In Fig. 3D, the rerank score that was carried out three times between native ligands and the 1GIH receptor was -53.20, -53.36, and -53.36, so the average value was -53.31, while the rerank score between 1,4-naphthoquinone and 6GU6 receptors was -110.44, -109.84, and -110.63, so the average value was-110.30. The difference in the mean rerank score, which was quite far between the native ligand and the 1,4-naphthoquinone, namely -53.31 and -110.30, indicates that the difference in activity was quite far. Therefore, 1,4-naphthoquinone did not effectively bind to 6GU6 receptors, which are CDK4 receptor proteins. This means 1,4-naphthoquinone could have little effect on inhibiting CDK4 activity in the cell cycle. The results of the rerank score were quite different between the native ligand and 1,4-naphthoquinone. The 6GUC receptor had the closest mean rerank score between native ligand A and 1,4-naphthoquinone, which were -90.55 and -54.66. Meanwhile, the farthest mean of rerank score occurred at the 6GU6 receptor, which was -114.05 and -48.09.

Fig. 4 is a description of the amino acid interactions that occur in native ligands and 1,4-naphthoquinone at each receptor, namely the 6GU6 receptor (Fig. 4A-B), the 6GUC receptor (Fig. 4C-F), and the 1GIH receptor (Fig. 4G-H). The types of interactions that occur, hydrogen bonding and steric interactions, and the distance and ligand groups are summarized in Table 2.

The same amino acid was not found between the 1,4-naphthoquinone and the native ligand at the 6GU6 receptor and the 1GIH receptor in all interactions. This indicates that there was no bond formed between 1,4-naphthoquinone and the 6GU6 receptor and the 1GIH receptor. So that there was no effect on CDK1 and CDK2 activity in the cell cycle when 1,4-naphthoquinone was given.

Meanwhile, similar amino acid bonds were found in Lys 33 (A) at 1,4-naphthoquinone in steric interactions and native ligand A in hydrogen and steric interactions at 6GUC receptors. In addition, the amino acid bonds in the form of Lys 33 (C) in 1,4-naphthoquinone were found in steric interactions and native B ligands in hydrogen interactions at the 6GUC receptor. This indicates that there was still sufficient potential for slight amino acid-binding between 1,4-naphthoquinone the 6GUC receptor, which is a receptor for CDK2 in the cell cycle, even though it only forms a weak bond, which may not have much effect in influencing CDK2 activity in the cell cycle.

Toxicity prediction

Based on Table 3, the prediction results of toxicity used parameters like lethal dose 50% (LD_{50}), toxicity class, AMES toxicity, hepatotoxicity, and skin sensitization. 1,4-Naphthoquinone has an LD_{50} = 190 mg/kg, including the class 3 toxicity group, which means that 1,4-naphthoquinone is estimated to have a toxic dose when administered as much as 190 mg/kg to experimental animals. This compound is predicted to cause toxicity to bacteria by showing "yes" to AMES toxicity and giving sensitivity to the skin but not causing hepatotoxicity.

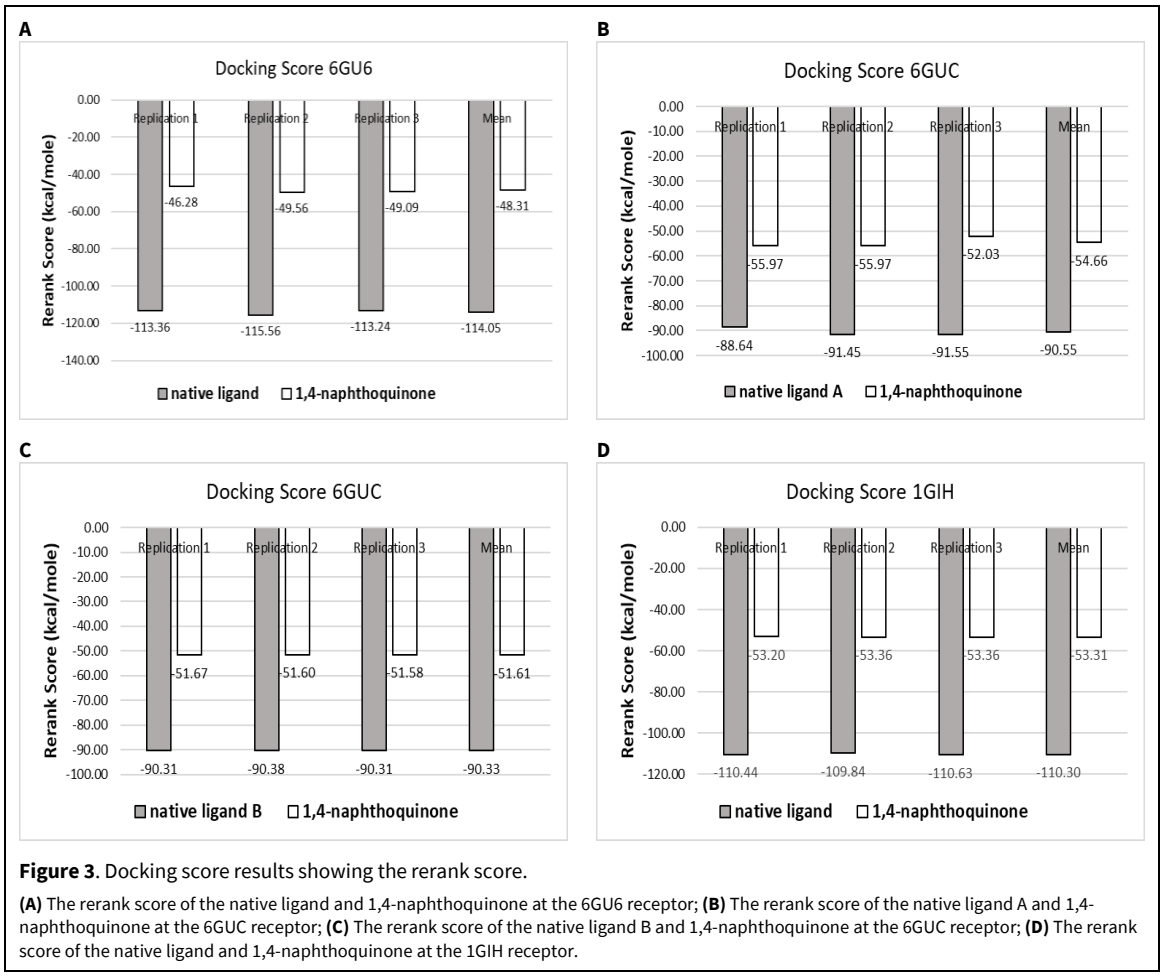

Figure 3. Docking score results showing the rerank score.

(A) The rerank score of the native ligand and 1,4-naphthoquinone at the 6GU6 receptor; **(B)** The rerank score of the native ligand A and 1,4-naphthoquinone at the 6GUC receptor; **(C)** The rerank score of the native ligand B and 1,4-naphthoquinone at the 6GUC receptor; **(D)** The rerank score of the native ligand and 1,4-naphthoquinone at the 1GIH receptor.

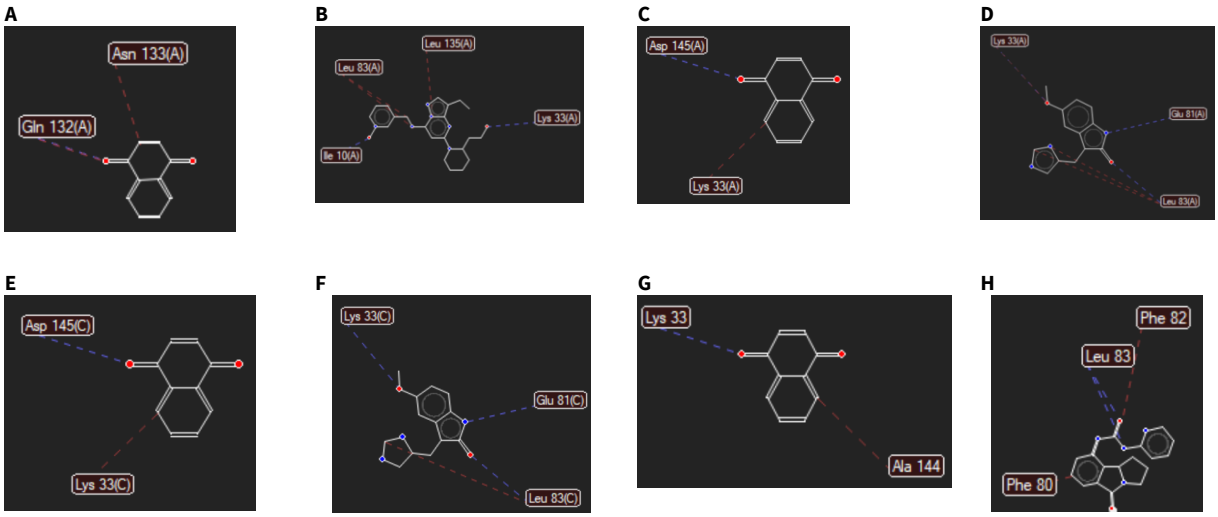

Figure 4. Two dimensions of amino acid interactions on native ligands and 1,4-naphthoquinone.

1,4-naphthoquinone **(A)** and native ligand **(B)** at the 6GU6 receptor. 1,4-naphthoquinone **(C,E)**, Native ligand A **(D)**, and Native ligand B **(F)** at the 6GUC receptor. 1,4-naphthoquinone **(G)** and native ligand **(H)** at the 1GIH receptor. Blue color: hydrogen bonds. Red color: steric interactions.

Table 2. Profile of amino acids and functional groups involved in hydrogen bonding and steric interactions.

Compounds	Receptor	Hydrogen bonding			Steric interactions		
		Amino acid	Distance (Å)	Ligand group	Amino acid	Distance (Å)	Ligand group
1,4-Naphthoquinone	6GU6	Gln 132(A)	3.24	Atomic O number 0	Gln 132(A)	3.24	Atomic O number 0
					Asn 133(A)	2.94	Atomic C number 8
Native ligand	6GU6	Ile 10(A)	2.83	Atomic O number 19	Leu 135(A)	3.18	Atomic N number 9
		Lys 33(A)	3.03	Atomic O number 28	Leu 83(A)	2.90	Atomic N number 11
						3.15	Atomic C number 12
1,4-Naphthoquinone	6GUC	Asp 145(A)	2.61	Atomic O number 0	Lys 33(A)	3.01	Atomic C number 6
Native ligand A	6GUC	Leu 83(A)	3.03	Atomic O number 8	Leu 83(A)	3.11	Atomic N number 0
		Glu 81(A)	3.03	Atomic N number 9	Leu 83(A)	3.10	Atomic C number 1
		Lys 33(A)	2.98	Atomic O number 14	Lys 33(A)	3.15	Atomic O number 14
1,4-Naphthoquinone	6GUC	Asp 145(C)	2.6	Atomic O number 0	Lys 33(C)	3.05	Atomic C number 6
Native ligand B	6GUC	Leu 83(C)	3.04	Atomic O number 8	Leu 83(C)	3.18	Atomic C number 1
		Glu 81(C)	3.01	Atomic N number 9			
		Lys 33(C)	3.08	Atomic O number 14			
1,4-Naphthoquinone	1GIH	Lys 33	3.05	Atomic O number 0	Ala 144	3.02	Atomic C number 7
Native ligand	1GIH	Leu 83	2.60	Atomic N number 14	Phe 80	2.99	Atomic C number 9
			2.74	Atomic O number 21	Phe 82	3.13	Atomic O number 21

: The amino acid from compound 1,4-naphthoquinone is the same as the native ligand.

Table 3. Toxicity prediction used Protox II online and pkCSM online tool.

Compound	LD_{50} (mg/kg)*	Toxicity group*	AMES toxicity**	Hepatotoxicity**	Skin sensitization**
1,4-Naphthoquinone	190	3	Yes	No	Yes

*Protox II Online Tool; **pkCSM Online Tool

DISCUSSION

Colorectal cancer (CRC) is a malignancy that grows in the large intestine (colon) and rectum by slowly growing polyps (ACS, 2017). Medical therapy such as surgery, which is the main action in treating CRC, as well as chemotherapy and radiotherapy, can be used before and after surgery. The use of chemotherapy for colorectal cancer at an advanced stage can improve the quality of life of cancer patients, although it can cause cancer cell resistance and other side effects (Monson et al., 2013).

Fig. 2 shows that as the dose of SPE increases, the expression of Ki-67 decreases more. This shows that the main compound 1,4-naphthoquinone in SPE can reduce the proliferation of large cancer cells. According to research by Zhang et al. (2018), 1,4-naphthoquinone effectively inhibits the proliferation of breast cancer cells and could be a promising anticancer agent. 1,4-naphthoquinone derivatives significantly inhibited gastric cancer cell survival by inducing apoptosis and cell cycle arrest in the G2/M phase in AGS (gastric cancer cell line) cells by stimulating ROS generation, leading to subsequent activation of the MAPK, Akt, and signaling pathways STAT3 (Wang et al., 2019).

Naphthoquinone derivatives induce cancer cell apoptosis through ROS-dependent mechanisms (Liu et al., 2018). Kayashima et al. (2009) compound 1,4-naphthoquinone in human colon cancer cells (HCT116) showed that higher doses result in lower cancer cell proliferation values. In addition, it was also found in the research of Wang et al. (2019) that the higher the dose of 1,4-naphthoquinone and its derivatives are given, the rate of gastric cancer cell proliferation decreased, marked by a decrease in the viability of cancer cells.

Rerank score or bond energy is the total energy of all the bonds, which means the amount of energy needed to make the ligand-receptor interaction occur (Guedes et al., 2014). The smaller the value of the bond energy obtained, the more stable the bond, and the more stable the ligand bond with the receptor, the greater activity of the compound. Based on Table 2, the results of the Rerank Score on all native ligands showed values that were smaller than those of 1,4-naphthoquinone compounds. This means the native ligand at the 6GU6, 6GUC, and 1GIH receptors have a higher affinity than 1,4-naphthoquinone compounds. 1,4-Naphthoquinone compounds cannot interact directly with the CDK/cyclin complex, so they do not have the potential to become CDK1, CDK2, and CDK4 inhibitors. Based on the significant difference between the rerank score of the native ligand and the test compound. Meanwhile, the amino acid-binding between the receptor and the test compound does not have a significant similarity with the original ligand. Only the Lys 33 (A) amino acid in CDK2 in steric interactions has similarities between the native ligand and the compound, is not strong enough to form a bond between the compound as a ligand and the receptor.

Based on Prachayasittikul et al. (2014), the 1,4-naphthoquinone produces reactive oxygen species, leading to apoptosis and DNA damage, causing cytotoxicity to the cancer cells. Quinone-based anti-dicancer drugs inhibit the enzyme DNA topoisomerase II required for chromosome condensation, DNA replication, and segregation. By inhibiting this enzyme, we can reduce cell proliferation activity. Based on Table 3, predicting the toxicity using LD_{50} obtained a value of 190 mg/kg, indicating that the bigger the score, the smaller the toxic effect, and it is predicted that 1,4-naphthoquinone is not hepatotoxic, so it could not affect liver function.

CONCLUSION

Sisyrinchium palmifolium L. ethanolic extract with the main compound 1,4-naphthoquinone could decrease Ki-67 expression by *in vivo*, which could induce a decrease in epithelial cells proliferation in colon cancer, but has no potential as an inhibitor activity of CDK1, CDK2, and CDK4 by *in silico* that are overexpressed in colon cancer.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

The author would like to thank the State Islamic University of Maulana Malik Ibrahim Malang, which has provided research funds to the prospective lecturer program in accordance with the Decree of the Chancellor of the State Islamic University of Maulana Malik Ibrahim Malang, Indonesia (number 1405 of 2020).

REFERENCES

ACS - American Cancer Society (2017) Colorectal Cancer Facts & Figure 2017-2019. American Cancer Society, pp. 1–36.

Afiati, Bethy S, Hernowo (2013) Relationship of Ki-67 expression with histopathological grading of liposarcoma. Bandung Med Book 45(3): 187–191

Annisa R, Hendradi E, Yuwono M (2020) Analysis of 1,4-naphthoquinone in the Indonesian medical plant from extract *Eleutherine palmifolia* (L.) Merr by UHPLC. IOP Conf Ser: Earth Environ Sci 456: 012020.

Grivennikov SI (2013) Inflammation and colorectal cancer: colitis-associated neoplasia. Semin Immunopathol 35(2): 229–244.

Guedes IA, Magalhaes CSD, Dardenne LE (2014) Review: receptor ligand molecular docking. Biophys Rev 6: 75–87.

Hwang D, Jo H, Kim JK, Lim YH (2017) Oxyresveratrol-containing *Ramulus mori* ethanol extract attenuates acute colitis by suppressing inflammation and increasing mucin secretion. J Funct Food 35: 146–158.

IARC - International Agency for Research on Cancer (2018) Latest global cancer data: Cancer burden rises to 18.1 million new cases and 9.6 million cancer deaths in 2018. World Health Organization, p. 1–3.

Kayashima T, Mori M, Yoshida H, Mizushina Y, Matsubara K (2009) 1,4-Naphthoquinone is a potent inhibitor of human cancer cell growth and angiogenesis. Cancer Lett 278(1): 34–40.

Kuntorini EM, Dewi M, Misrina (2016) Anatomical structure and antioxidant activity of red bulb plant *Eleutherine americana* on different plant age. Biodiversitas 17(1): 229–233.

Li X, Ohtsuki T, Koyano T, Kowithayakorn T, Ishibashi M (2008) New Wnt/β-catenin signaling inhibitors isolated from *Eleutherine palmifolia*. Chem Asian J 4: 540–547.

Liu C, Shen GN, LuoYH, Piao XJ, Jiang XY, Meng LQ, Wang Y, Zhang Y, Wang JR, Wang H, Xu WT, Li JQ, Liu Y, Wu YQ, Sun HN, Han YH, Jin MH, Cui YD, Fang NZ, Jin CH (2018) Novel 1,4-naphthoquinone derivatives induce apoptosis via ROS-mediated p38/MAPK, Akt and STAT3 signaling in human hepatoma Hep3B cells. Int J Biochem Cell Biol 96: 9–19.

Marmol I, Sanchez C, Pradilla DA, Cerrada E, Rodriguez YMJ (2017) Colorectal carcinoma: a general overview and future perspectives in colorectal cancer. Int J Mol Sci 18(1): 197.

Mikhail S, Albanese C, Pishvaian MJ (2015) Cyclin-dependent kinase inhibitors and the treatment of gastrointestinal cancers. Am J Pathol 185(5): 1185-1197.

Monson JRT, Weiser MR, Buie WD, Chang GJ, Rafferty JF (2013) Practice parameters for the management of rectal cancer. Dis Colon Rectum 56(5): 535–550.

Muti'ah R, Anik L, Arief S, Rahmi A, Abdul H, Wirda A, Retno S (2018) Activity of inhibit the cell cycle and induct apoptosis in HeLa cancer cell with combination of sabrang onion (*Eleutherine palmifolia* (L.) Merr) and starfruit mistletoe (*Macrosolen cochinchinensis* (Lour.) Tiegh). J App Pharm Sci 8(10): 122–128.

Muti'ah R, Wahyi YF, Riza AS, Rahmi A, Risma A, Yen YAI, Tias PG, Anik L (2020) *Eleutherine palmifolia* (L.) Merr. extract increases the crypts and caspase-3 expression in colitis-associated colon cancer model. Indones J Pharm 31(4): 257–265.

Pattanayak SP, Sunita P, Mazumder PM (2014) Restorative effect of *Dendrophthoe falcata* (L.f.) ettingsh on lipids lipoproteins, and lipid metabolizing enzymes in DMBA-induced mammary gland carcinogenesis in Wistar female rats. Comp Clin Pathol 23: 1013–1022.

Prachayasittikul V, Pingaew R, Worachartcheewan A, Nantasenamat C, Prachayasittikul S, Ruchirawat S, Prachayasittikul V (2014) Synthesis, anticancer activity and QSAR study of 1,4-naphthoquinone derivatives. Eur J Med Chem 84: 247–263.

Syarif H (2014) The effectiveness of dayak onion extract (*Eleutherine palmifolia* (L) Merr.) in inhibiting the growth of *Escherichia coli* bacteria. Final Project of Bachelor of Medicine. Jakarta, Indonesia.

Tadbir AA, Pardis S, Ashkavandi ZJ, Najvani AD, Ashraf MJ, Taheri A, Zadeh MA, Sardari Y (2012) Expression of Ki67 and CD105 as proliferation and angiogenesis markers in salivary gland tumors. Asian Pac J Cancer Prev 13(10): 5155–5159.

Wang H, Luo YH, Shen GN, Piao XJ, Xu WT, Zhang Y, Wang JR, Feng YC, Li JQ, Zhang Y, Zhang T, Wang SN, Xue H, Wang HX, Wang CY, Jin CH (2019) Two novel 1,4-naphthoquinone derivatives induce human gastric cancer cell apoptosis and cell cycle arrest by regulating reactive oxygen species-mediated MAPK/Akt/STAT3 signaling pathways. Mol Med Rep 20(3): 2571–2582.

Zhang Q, Dong J, Cui J, Huang G, Meng Q, Li S (2018) Cytotoxicity of synthesized 1,4-naphthoquinone oxime derivatives on selected human cancer cell lines. Chem Pharm Bull 66(6): 612–619.

AUTHOR CONTRIBUTION:

Contribution	Muti'ah R	Endharti AT	Wafi MF
Concepts or ideas	x	x	x
Design	x		x
Definition of intellectual content	x	x	x
Literature search	x	x	x
Experimental studies	x		x
Data acquisition	x	x	x
Data analysis	x	x	x
Statistical analysis	x	x	x
Manuscript preparation	x	x	x
Manuscript editing	x		x
Manuscript review	x	x	x

Citation Format: Muti'ah R, Endharti AT, Wafi MF (2022) Effect of 1,4-naphthoquinone from *Sisyrinchium palmifolium* L. extract on *in vivo* Ki-67 expression and *in silico* CDK1, CDK2, CDK4 on colitis-associated colon cancer. J Pharm Pharmacogn Res 10(4): 595–604. https://doi.org/10.56499/jppres21.1321.10.4.595