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Original Article

COMPUTATIONAL INVESTIGATION OF THE INHIBITION OF CHK1 AND WEE1 PROTEINS BY CHEMICAL CONSTITUENTS OF AMARANTHUS GANGETICUS

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ABSTRACT

Objective: This study aimed to explore an alternative compound capable of inhibiting CHK1 (Checkpoint Kinase 1) and WEEl proteins in breast cancer using natural compounds derived from red spinach (*Amaranthus gangeticus*).

Methods: The experiment used SMILES and 3D structure of red spinach, PASS Online for biology activity, Lipinski's rule of five for physicochemical properties predictions, as well as validation, and molecular docking of active compounds.

Results: The results showed that CHK1 and WEE1 docking validation had RMSD values of 1.5 Å and 0.634 Å, respectively. The compounds 3,4,5-Trihydroxybenzoic acid, 3,5-Dimethoxy-4-hydroxybenzoic acid, 2,3,7,8-Tetrahydroxy-chromeno[5,4,3-cde]chromene-5,10-dione, 3-(3,4-Dihydroxycinnamoyl)quinic acid, 3-Methoxy-4-hydroxycinnamic acid, 4-Hydroxy-3,5-dimethoxycinnamic acid, 3-Phenylacrylic acid, 4′,5,7-Trihydroxyflavone, Catechin, and Quercetin exhibited favourable binding affinity values, molecular interactions, and predicted inhibitory effects against CHK1 by interacting with key residues LEU A 15, VAL A 23, and LEU A 137, and against WEE1 through interactions with GLU A 377, ILE A 305, VAL A 313, ALA A 326, and PHE A 433. While these findings highlight promising inhibitory potential, further *in vitro* and *in vivo* validation is needed to confirm the computational findings.

Conclusion: This study found that the active compounds of red spinach could be used as a functional inhibitor of CHK1 and WEE1.

Keywords: Breast cancer, Red spinach (*Amaranthus gangeticus*), CHK1, WEE1, *In silico*

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INTRODUCTION

Cancer is an illness resulting from the abnormal proliferation of cells in the tissues of the body due to mutations of genetic. It is a global health problem, and a leading cause of death worldwide, with breast cancer being the second most prevalent reason of death among women. In Indonesia, statistics provided by the Global Cancer Observatory showed that there were 65,858 (16.6%) new cases of breast cancer out of 396,914 with a total of 22,430 deaths (9.6%) in 2020 [1, 2].

Chemical drugs, such as doxorubicin, often cause resistance, specifically in T47D breast cancer cells, while long-term use leads to toxicity and side effects such as hair loss and low blood pressure. Consequently, natural ingredients are preferred due of their improved safety profile [3, 4].

Breast cancer arises from genetic mutations leading to uncontrolled cell proliferation, and it remains one of the leading causes of cancer-related death in women [5]. The associated risk factors include age at menarche, obesity, duration of using hormonal contraception, smoking, and history of breastfeeding [6]. High breast cancer incidence is partly attributed to limited awareness of risk factors and the importance of early examination [7].

A previous study showed that low vegetable consumption was associated with heightened cancer susceptibility [8]. Based on an *in vitro* investigation, water and ethanol extract from red spinach (*Amaranthus gangeticus*) had cytotoxic activity against several lines of cells derived from cancerous tissues [9]. Production demand for the plant reached 148,288 tons. The antioxidant content of red spinach is theorized to have anticancer (anti-mitotic) properties. Notably, kaempferol, a flavonoid present in red spinach, has been reported to suppress proliferation and induce cell cycle arrest, apoptosis, and DNA damage in breast cancer cells. However, direct inhibition of WEE1 or CHK1 kinases by kaempferol has not been demonstrated. Further research is needed to clarify its precise molecular targets and compare its efficacy with clinically used CHK1/WEE1 inhibitors [10-12].

CHK1 (Checkpoint Kinase 1) is a protein kinase that functions as a central genome surveillance system for replication and responding to DNA damage. The activation occurs when there is DNA damage, helping maintain genome stability. CHK1 inhibition slows the S phase by arresting cell replication, preventing entry into the M phase. It also activates WEE1 and MYT1, to preserve CDK1 in a dormant condition. In addition, CHK1plays an important role for controlling cycle of cell [13].

WEE1 is an essential component in G2/M checkpoint, regulating the replication and maintenance of DNA. The inhibition blocks CDK1 activity by initiating phosphorylation at Thr14 and Tyr15, activating the G2-M phase. DNA damage will cause WEE1 kinase to inactivate CDK1/2, thereby preventing cells from continuing mitosis by maintaining G2 arrest [13].

CHK1 and WEE1 inhibition synergistically affects cycle of cell's stability, triggering damage of DNA and promoting progressing death from cell rapidly. The inhibition of WEE1 makes the replication fork in cancer cells stop and break down. Co-inhibition of these two proteins blocks the ability of cells to repair damage, thereby inducing apoptosis or cell death [14].

Several CHK1 and WEE1 inhibitors are currently available on the market, including Adavosertib (AZD1775), a selective WEE1 inhibitor, and Prexasertib, a CHK1 inhibitor. Adavosertib has demonstrated potential in various clinical trials, often in combination with DNA-damaging agents such as chemotherapy. However, it is associated with significant side effects, including hematological toxicity, gastrointestinal distress, and fatigue [15, 16]. Similarly, Prexasertib induces DNA damage and apoptosis but can lead to severe myelosuppression, limiting its clinical application. Given these limitations, the search for novel, less toxic inhibitors remain crucial,

highlighting the potential of natural compounds such as kaempferol as alternative candidates for CHK1/WEE1 inhibition [17, 18].

Computational method is used for predicting interactions between two molecules, producing a binding model that can predict the mechanism of action for active compounds [19]. Although in silico analysis is crucial, further *in vitro* stages on red spinach extract are necessary to determine the activity as an anticancer candidate by inhibiting CHK1 and WEE1 proteins.

MATERIALS AND METHODS

Tools

Hardware: Computer with specification of Windows 11 Pro 64-bit (Build 22621.608), 12th Gen Intel® Core™ i7-12700H CPU @2.70 GHz (20CPUs), 16.0 GB memory.

Software: Molecular docking was conducted using AutoDock Tools 1.5.7 and AutoDock Vina 1.1.2 [20]. Visualization of docking interactions was performed using Biovia Discovery Studio Visualizer 4.5 [21] and PyMOL 2.5 [22]. Physicochemical properties were assessed using Lipinski's rule of five via SCFBio-IITD (http://www.scfbio-iitd.res.in) [23]. Biological activity prediction was conducted using PASS Online via (http://way2drug.com/PassOnline/) [24]. Protein structures were obtained from the Protein Data Bank (PDB)via (www.rcsb.org) [25]. Chemical compound data were retrieved from PubChem via (https://pubchem.ncbi.nlm.nih.gov/) [26].

Bioactive compounds of red spinach (*Amaranthus gangeticus*)

Based on several studies conducted by Kalanjati *et al.* (2014), Sarker and Ercisli (2022), as well as Sarker and Oba (2020, 2021), *Amaranthus gangeticus* has been identified as a rich source of bioactive compounds with significant nutritional and antioxidant properties. The active compounds included 3,4,5-Trihydroxybenzoic acid, 4-Hydroxy-3-methoxybenzoic acid, 3,5-Dimethoxy-4-hydroxybenzoic acid, 4-Hydroxybenzoic acid, 2-Hydroxybenzoic acid, 2,3,7,8-Tetrahydroxychromeno[5,4,3cde]chromene-5,10-dione, 3,4-Dihydroxybenzoic acid, 2,4-Dihydroxybenzoic acid, 2,5-Dihydroxybenzoic acid, 3,4-Dihydroxy-trans-cinnamate, 3-(3,4-Dihydroxycinnamoyl)quinic acid, 4-Hydroxycinnamic acid, 3-Methoxy-4-hydroxy cinnamic acid, 3-Hydroxycinnamic acid, 4-Hydroxy-3,5-dimethoxy cinnamic acid, 3-Phenylacrylic acid, Quercetin-3-O-glucoside, Quercetin-3-O-galactoside, Quercetin-3-O-rutinoside, Myricetin-3-O-rutinoside, 4′,5,7-Trihydroxyflavone, Kaempferol-3-O-rutinoside, Neoxanthin, Violaxanthin, Lutein, Zeaxanthin, β-carotene, Ascorbic acid, folic acid, Catechin, Narigenin, Quercetin, Betanin, Isobetanin, and Betalains [27-30].

Biology activity prediction using pass online (prediction of activity spectra substances)

Biological activity predictions were carried out using the PASS Online web server (http://way2drug.com/PassOnline/), which predicts pharmacological properties based on structure-activity relationships (SAR) using machine learning and Bayesian models [24]. SMILES representations of the compounds were obtained from PubChem before input into PASS Online [26].

Physiochemistry prediction (Lipinski's rule of five)

Physiochemistry predictions were analyzed using Lipinski's Rule of Five [23], implemented via the SCFBio-IITD web server (http://www.scfbio-iitd.res.in). The 3D molecular structures were obtained from PubChem, and analysis included molecular weight, hydrogen bond donors/acceptors, and logP values [26].

Proteins, native ligand, ligand preparation

Proteins were obtained from protein data bank (www.rcsb.org) [25], using CHK1 (2YEX resolution 1.30 Å) and WEE1 (1X8B resolution 1.81 Å). Proteins preparations were done using AutodockTools1.5.7 [20], with a 3D structure of CHK1 (2YEX) and WEE1 (1X8B) by removing water, polarizing the proteins, and adding Kollman charges. Proteins preparation using AutodockTools 1.5.7 determined the grid box in the area known to be the active site of the protein. The grid box was determined by setting the location of the box parameters and selecting the size using spacing (angstrom). In protein CHK1 (PDB ID: 2YEX), the results of setting the grid box are as follows: center_x = 1.229 center_y = 37.708 center_z = 10.367 with spacing (angstrom) determined by 1 Å. In protein WEE1 (PDB ID: 1X8B), the results of setting the grid box are as follows: center_x = 3.985 center_y = 53.976 center_z = 25.971, with spacing (angstrom) determined by 1 Å. Native ligand preparation from a compound of CHK1 native ligand, namely 5-Methyl-8-(1H-Pyrrol-2-YL)[1,2,4]Triazolo[4,3-A]Quinolin-1(2H)-One and a compound of WEE1 native ligand namely9-Hydroxy-4-Phenylpyrrolo[3,4-C]Carbazole-1,3(2H,6H)-Dione was performed with removing water, selecting the torsion, and setting the number of torsions. Furthermore, the ligand was obtained from website PubChem (https://pubchem.ncbi.nlm.nih.gov/) in sdf form [20]. Stages in ligand preparation using Biovia Discovery Studio Visualizer 4.5 started with converting the file into pdb form [21].

Docking validation

Validation was completed by docking the proteins with a compound of CHK1 native ligand namely 5-Methyl-8-(1H-Pyrrol-2-YL)[1,2,4]Triazolo[4,3-A]Quinolin-1(2H)-One and a compound of WEE1 native ligand namely 9-Hydroxy-4-Phenylpyrrolo[3,4-C]Carbazole-1,3(2H,6H)-Dione, after setting the grid box, size, and centre coordinates. The docking procedure used Autodock Vina 1.1.2 [20], with each conformation outcome split and then visualized using Pymol 2.5 [22].

Ligand-protein docking

Molecular docking was conducted using the ligand and protein that had been prepared initially. Ligand was prepared by selecting a set number of torsions, then the grid box size, and centre coordinates were set. Molecular docking procedure was completed by using Autodock Vina 1.1.2 [20].

Visualization of docking result

Biovia Discovery Studio Visualizer 4.5 was used for visualizing the docking result by examining the ligand interaction and 2D diagram [21].

RESULTS AND DISCUSSION

Biology activity prediction using pass online

PASS Online test, commonly used for predicting biology activity with 90% accuracy, has Pa (Probable active) and Pi (Probable inactive) numbers, Pa and Pi activity is considered possible for certain active compounds. The values of Pa and Pi are between 0.000-1.000 in general [31]. The results are shown in fig. 1.

The criteria used to determine the Pa (Probable activity) value are as follows: (1) Pa>0.7 suggests an analogous activity to a drug compound, (2) 0.5 Pa<0.7, indicates different forms, with less possibility of showing analogous activity, and (3) Pa<0.5, implies a lack of analogous activity. Therefore, PASS Online prediction activity is considered an intrinsic property of the compound [31].

Nine compounds had a Pa value<0.5, suggesting a lack of activity as a promising breast cancer drug candidate. When the Pa value is<0.5, the tested compound does not show analogous activity to the drug such as 4-Hydroxybenzoic acid,2-Hydroxybenzoic acid, 3,4-Dihydroxybenzoic acid, 2,4-Dihydroxybenzoic acid, 2,5-Dihydroxybenzoic acid, Folic Acid, Betanin, Isobetanin, Betalains.

Based on the results, 35 compounds were predicted to have antioxidant activity, 30 demonstrated antineoplastic activity, 20 compounds had cancer-associated disorders treatment activity, 27 compounds were predicted to have antineoplastic (breast cancer) activity, five had breast cancer-resistant protein inhibitor activity, and one had WEE-1 tyrosine kinase inhibitor activity [31].

These computational predictions align with previous studies demonstrating the anticancer potential of *Amaranthus gangeticus* extracts. For instance, an aqueous extract of *A. gangeticus* inhibited the proliferation of liver (HepG2) and breast (MCF-7) cancer cell lines, with IC_{50} values of 93.8 µg/ml and 98.8 µg/ml, respectively. *In vivo* studies on rats indicated that supplementation with *A.*

gangeticus extract reduced tumour marker enzyme activities, suggesting potential chemopreventive properties [9]. Additionally, gold nanoparticles synthesized using phenolic-rich fractions of *A. gangeticus* exhibited potent anticancer activity against HeLa cells *in vitro* [32]. Additionally, the selection of key compounds was based not only on PASS Online predictions but also on docking scores and physicochemical properties, including Lipinski's rule assessment, to ensure their potential as drug candidates.

Physiochemistry prediction using lipinski's rule of five

The Lipinski's rule of five is used to predict physicochemical properties of a compound. This website is used for assessing the bioavailability of oral potential of drug [33]. The results are shown in table 1.

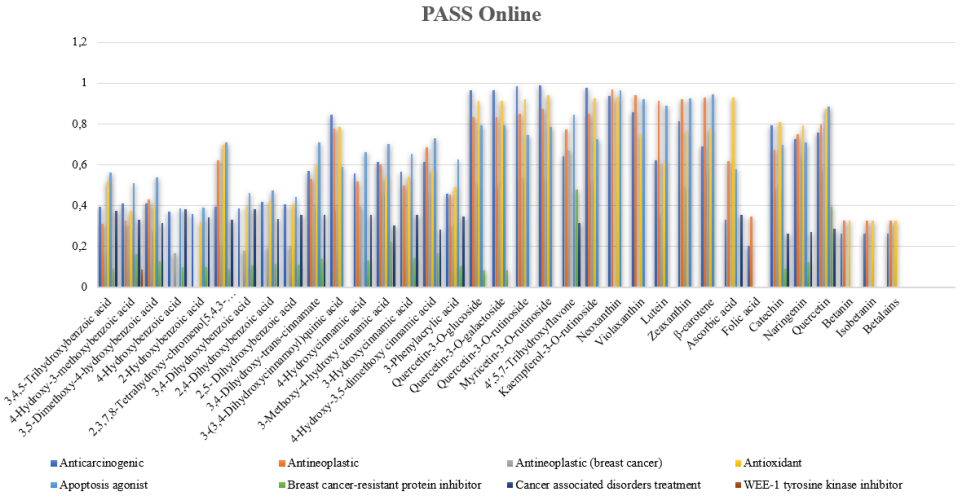


Fig. 1: Biology activity prediction from *Amaranthus gangeticus* using PASS online

Table 1: Physiochemistry prediction from *Amaranthus gangeticus* using lipinski's rule of five

Active compound	Molecular weight	Log P	Hydrogen bond donor	Hydrogen bond acceptor	Molar refractivity	Number of violations
3,4,5-Trihydroxybenzoic acid	170	0.50	4	5	38.39	1
4-Hydroxy-3-methoxybenzoic acid	168	1.10	2	4	41.62	0
3,5-Dimethoxy-4-hydroxybenzoic acid	198	1.11	2	5	48.17	0
4-Hydroxybenzoic acid	138	1.10	2	3	35.07	1
2-Hydroxybenzoic acid	138	1.10	2	3	35.07	1
2,3,7,8-Tetrahydroxy-chromeno[5,4,3-cde]chromene-5,10-dione	302	1.24	4	8	68.45	0
3,4-Dihydroxybenzoic acid	154	0.80	3	4	36.73	1
2,4-Dihydroxybenzoic acid	154	0.80	3	4	36.73	1
2,5-Dihydroxybenzoic acid	154	0.80	3	4	36.73	1
3,4-Dihydroxy-trans-cinnamate	180	1.19	3	4	46.44	0
3-(3,4-Dihydroxycinnamoyl)quinic acid	354	-0.64	6	9	82.52	1
4-Hydroxycinnamic acid	164	1.49	2	3	44.78	0
3-Methoxy-4-hydroxy cinnamic acid	194	1.50	2	4	51.33	0
3-Hydroxycinnamic acid	194	0.98	3	4	50.70	0
4-Hydroxy-3,5-dimethoxy cinnamic acid	224	1.51	2	5	57.88	0
3-Phenylacrylic acid	148	1.78	1	2	43.11	0
Quercetin-3-O-glucoside	464	-0.73	8	12	106.27	2
Quercetin-3-O-galactoside	464	-0.73	8	12	106.27	2
Quercetin-3-O-rutinoside	610	-1.88	10	16	137.49	4
Myricetin-3-O-rutinoside	626	-2.17	11	17	139.16	4
4′,5,7-Trihydroxyflavone	270	2.42	3	5	70.81	0
Kaempferol-3-O-rutinoside	594	-1.58	9	15	135.83	4
Neoxanthin	312	-0.05	5	6	77.14	0
Violaxanthin	312	-0.05	5	6	77.14	0
Lutein	568	10.40	2	2	184.10	3
Zeaxanthin	568	10.55	2	2	184.17	3
β-carotene	536	12.60	0	0	181.39	3
Ascorbic acid	176	-1.41	4	6	35.26	1
Folic acid	441	-0.17	7	13	110.31	3
Catechin	290	1.55	5	6	72.62	0
Naringenin	272	2.51	3	5	70.19	0
Quercetin	302	2.01	5	7	74.05	0
Betanin	550	-2.73	8	15	125.42	3
Isobetanin	550	-2.73	8	15	125.42	3
Betalain	550	-2.73	8	15	125.42	3

Almost all red spinach active compounds meet the Lipinski rule of five, but there are some compounds that violate the rule because the compounds only met two of five requirements, which can cause drug bioavailability problems such as Quercetin-3-O-glucoside, Quercetin-3-

O-galactoside, Quercetin-3-O-rutinoside, Myricetin-3-O-rutinoside, Kaempferol-3-O-rutinoside, Lutein, Zeaxanthin, β-carotene, Folic acid, Betanin, Isobetanin, Betalain. These violations impact the permeability and solubility of the compounds, as they are highly hydrophilic, indicated by negative logP values and the hydrogen bond donor and acceptor values. As a result, these compounds struggle to cross lipid membranes and have poor solubility in lipids. Similarly, Lutein, Zeaxanthin, β-carotene, Betanin, Isobetanin, and Betalain also violate Lipinski's Rule, these compounds have molecular weights exceeding 500 Da, which further indicates that these compounds will have difficulty penetrating due to their large molecular size [33].

When a molecule violates one or more of these criteria, it is considered to have low bioavailability. The criteria including: molecular weight of the compound is below 500 Da, log P<5; hydrogen bond donors<5, hydrogen bond acceptors<10, and molar refractivity is 40-130 [34].

Docking validation

Docking validation result, Root mean Square Deviation (RMSD) value were computed using heavy atoms on CHK1 was 1.5 Å (fig. 2) and WEE1 was 0.634 Å (fig. 3), respectively, suggesting minimal error. The results of the molecular validation analysis were evaluated with RMSD value. Value below 2.00 A show that the molecular docking results are accurate. RMSD value close to zero is preferred as it guarantee greater dependability of ligand binding predictions and signifies a closer location of the ligand in binding site of protein [35].

Ligand-protein docking

Based on ligand-protein docking with three repetitions show that the standard deviation values from CHK1 ranged from 0.001-0.37, Prexasertib has 0.01, and native ligand has 0.006 and WEE1 ranged from 0.002-0.12, Adavosertib has 0.08, and native ligand has 0.02. Standard deviation is used to measure the distribution of data in sample, which provides information about how far the value in a sample are spread out from average. If the standard deviation value is high, it indicates that the value in the sample is widely spread from average. On the other hand, if the standard deviation value is low, it indicates that the value in the sample is close to the average, this indicates a good data. Based on docking analysis, the binding affinity values were obtained to see the binding affinity between ligand and protein [36]. The results are shown in fig. 4 and fig. 5.

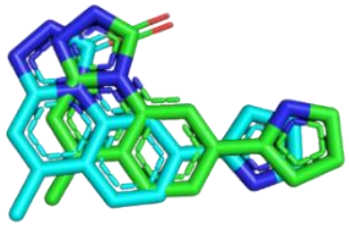


Fig. 2: Validation of native ligand (green) and ligand docking (blue) CHK1

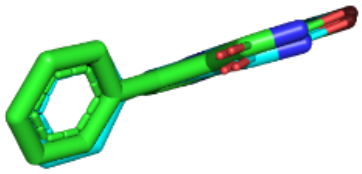


Fig. 3: Validation of native ligand (green) and ligand docking (blue) WEE1

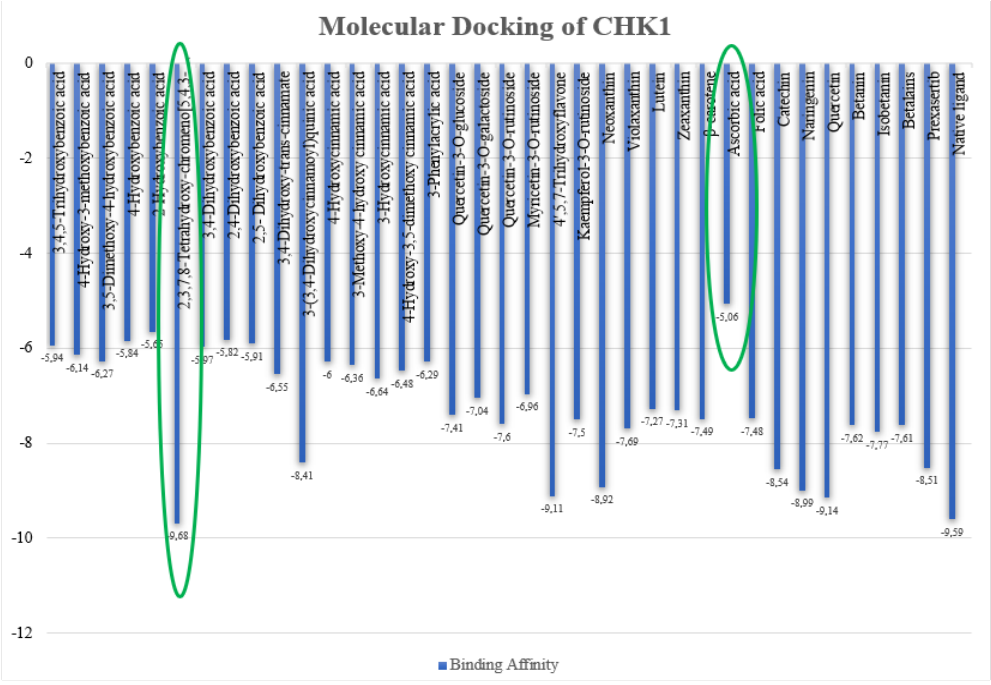


Fig. 4: Binding affinity values from the active compound of red spinach (*Amaranthus gangeticus*) from CHK1

CHK1 and WEE1 have the same strongest binding affinity namely 2,3,7,8-Tetrahydroxy-chromeno[5,4,3-cde]chromene-5,10-dione and the weakest binding affinity namely ascorbic acid. Binding affinity is a parameter in regulating the bond between ligand and receptor. The binding affinity values obtained for the active compound of red spinach ranged from-9.68 to-5.06 and-11.02 to-5.19 for CHK1 and WEE1. The stronger connection between the protein and the ligand, the lower the binding affinity value [36].

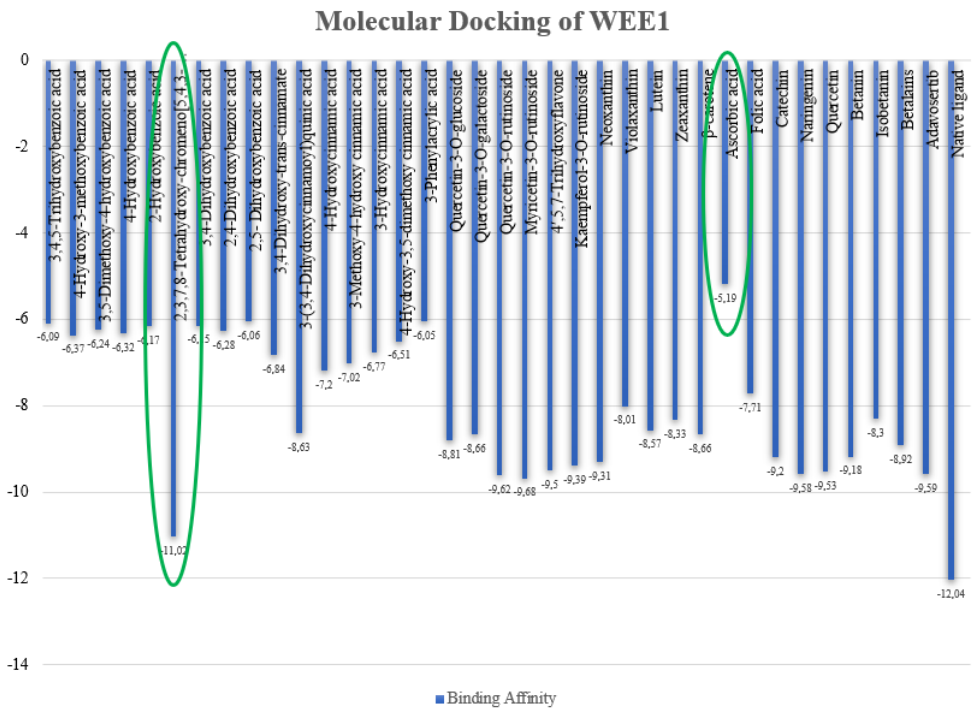
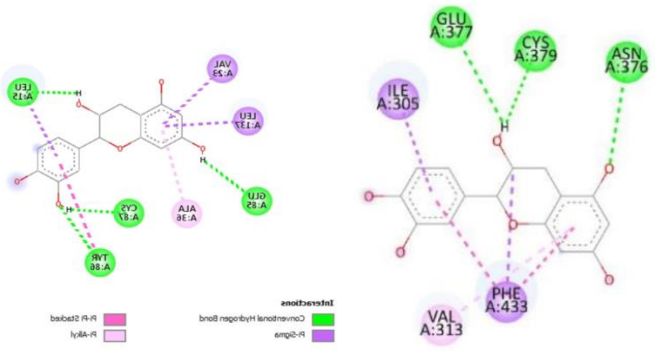


Fig. 5: Binding affinity values from the active compound of red spinach (*Amaranthus gangeticus*) from WEE1

Visualization experiment was used to observe the outcomes of docking for both the reference and experimental ligand with CHK1 protein. Docking visualization results between the active compound from red spinach, the native ligand, and the drug showed the amino acid residues formation. Between hydrogen bond of native ligand and reference, the same amino acid residue was obtained, namely SER A 147. In contrast, three identical amino acid residues were derived in the hydrophobic and other bonds, namely LEU A 15, LEU A 137, ALA A 36, and VAL A 23. Yin *et al.* (2019) identified potential CHK1 inhibitors with amino acid residues LEU A 15, VAL A 23, GLY A 90 and LEU A 137 in the hydrophobic bond and hydrogen GLU A 85 and CYS A 87 [37]. The results can be seen in Fig. 6.

Docking visualization results between the active compound of red spinach, native ligand, and drug indicated the types of interactions formed between WEE1 protein. The conserved binding interactions among the comparator and native ligand were derived in the hydrogen bonds, namely GLU A 377, TYR A 378, hydrophobic bonds, including ILE A 305, VAL A 313, ALA A 326, LYS A 328, VAL A 360, PHE A 433. Similarly, Squire *et al.* (2005) obtained hydrogen bonds, namely GLU A 377, as well as hydrophobic bonds of ILE A 305, VAL A 313, ALA A 326, and PHE A 433 [38].

On CHK1, five compounds, namely 3,4,5-Trihydroxybenzoic acid, 3-Methoxy-4-hydroxy cinnamic acid, 4-Hydroxybenzoic acid, 3-Phenyl acrylic acid, and 3-(3,4-Dihydroxy cinnamoyl)quinic acid, were found to have identical hydrophobic amino acid residues as native ligand and drug. Meanwhile, on WEE1, the native ligand, the comparator, and the results by Squire *et al.* (2005) share the same hydrophobic and hydrogen bonding with eleven active compounds. These include3,4,5-Trihydroxybenzoic acid, 3,5-Dimethoxy-4-hydroxybenzoic acid, 2,3,7,8-Tetrahydroxy-chromeno[5,4,3-cde]chromene-5,10-dione,3-(3,4-Dihydroxy-cinnamoyl)quinic acid, 3-Methoxy-4-hydroxy cinnamic acid, 4-Hydroxy-3,5-dimethoxy cinnamic acid, 4′,5,7-Trihydroxyflavone, Catechin, Quercetin, Betanin, and Isobetanin. Docking of red spinach compounds showed conventional and carbon-hydrogen bonds. An increased number of hydrogen bonds contributes to a more stable connection between the ligand and the protein. Both the hydrophobic and hydrogen bonds formed served as indicators of interaction intensity between the protein and ligand [35].



CHK1 **WEE1**

Fig. 6: Molecular docking results showing key hydrogen bond and hydrophobic interactions between CHK1, WEE1 and selected red spinach compounds

CONCLUSION

In conclusion, the validation of proteins CHK1 (2YEX) and WEE1 (1X8B) had RMSD 2 Å. Based on PASS Online test prediction, 26 compounds have an anticancer activity, while evaluation of drug-likeness suggested that 26 compounds have potential oral bioavailability according to Lipinski's rule of five. Furthermore, docking analysis showed that the active compound of red spinach had a binding pocket on LEU A 15, VAL A 23, and LEU A 137 signifying the inhibitory side of CHK1. There was also a binding pocket on GLU A 377, ILE A 305, VAL A 313, ALA A 326, and PHE A 433, implying the inhibitory side of WEE1. These findings suggest that red spinach compounds may serve as potential CHK1 and WEE1 inhibitors, warranting further *in vitro* and *in vivo* validation.

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AUTHORS CONTRIBUTIONS

Conceptualization: ASR, HSW, EDLX, NAZ; table Work: ASR, HSW, EDLX, NAZ, CA, TR; Supervision: ASR, HSW, EDLX, NAZ; Revisions: HSW, CA, TR; Writing and Editing: CA, TR; Proofreading: HSW, CA, TR.

CONFLICT OF INTERESTS

The author has no conflict of interest.

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