

THE POTENTIAL OF ACTIVE COMPOUNDS *Polyscias Scutellaria* AS INHIBITORS IN CERVICAL CANCER WITH VIRTUAL SCREENING APPROACH

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ABSTRACT

THE POTENTIAL OF ACTIVE COMPOUNDS *Polyscias Scutellaria* AS INHIBITORS IN CERVICAL CANCER WITH VIRTUAL SCREENING APPROACH Cervical cancer is a gynecologic disease that has a high enough malignancy that affects women. In cervical cancer, it is known that the overexpression of Bcl-2 protein is 14.3% in non-invasive cervical carcinoma and 65.2% in invasive cervical carcinoma. The Bcl-2 protein is an important regulator of growth and differentiation pathways. Bcl-2 has anti-apoptotic activity but permeabilization occurs through the mitochondrial pathway, triggering the release of cytochrome c, which then interacts with Apaf-1 for caspase-9 activation and apoptosis occurs. This study aims to determine the mechanism of inhibition of overexpression of Bcl-2 protein by bioactive compounds from the *Polyscias scutellaria* plant with a virtual screening approach through in silico studies. The in silico study was carried out with the stages of tracking molecular targets, preparation, optimization, simulation and analysis of docking results. The results of the docking analysis showed that the bioactive compounds of the mangkokan plant provided inhibitory activity with gibbs energy values of -6.07, -5.18, -5.43 and -6.02, respectively. Thus the bioactive compounds from the mangkokan plant have potential as Bcl-2 inhibitors in cervical cancer.

Key words : Cervical Cancer, *Polyscias scutellaria*, Virtual Screening, Docking Simulation

ABSTRAK

POTENSI SENYAWA AKTIF *Polyscias Scutellaria* SEBAGAI INHIBITOR KANKER SERVIKS DENGAN PENDEKATAN VIRTUAL SCREENING Kanker serviks adalah penyakit ginekologik yang memiliki tingkat keganasan yang cukup tinggi yang menyerang wanita. Pada kanker serviks diketahui over ekspresi protein Bcl-2 sebesar 14,3% pada karsinoma serviks non invasif dan 65,2% pada karsinoma serviks invasif. Protein Bcl-2 merupakan regulator penting dalam jalur pertumbuhan dan diferensiasi. Bcl-2 memiliki aktivitas anti-apoptosis tetapi melalui jalur mitokondria terjadi permeabilisasi sehingga memicu pelepasan sitokrom c, yang kemudian berinteraksi dengan Apaf-1 untuk aktivasi kaspase-9 dan terjadi apoptosis. Penelitian ini bertujuan untuk mengetahui mekanisme inhibisi ekspresi berlebih dari protein Bcl-2 oleh senyawa bioaktif dari tanaman *Polyscias scutellaria* (mangkokan) dengan pendekatan virtual screening melalui studi in silico. Studi in silico dilakukan dengan tahapan penelusuran target molekular, preparasi, optimasi, simulasi dan analisis hasil docking. Hasil analisis docking menunjukkan senyawa bioaktif tanaman mangkokan memberikan aktivitas inhibisi dengan nilai energi gibbs sebesar -6,07, -5,18, -5,43 dan -6,02 berturut-turut. Dengan demikian senyawa bioaktif dari tanaman mangkokan memiliki potensi sebagai inhibitor Bcl-2 pada kanker serviks.

Kata kunci : Kanker Serviks, *Polyscias scutellaria*, Virtual Screening, Simulasi Docking

INTRODUCTION

Cancer is a disease involving the abnormality in cell growth, including women's reproductive systems such as cervix. Cervical

cancer is a gynaecological disease is quite deadly. The data from Globocan *International Agency for Research on Cancer* (IARC) in 2012

reported that cervical cancer is the world's fourth most deadly disease (Ferlay *et al.*, 2015). The Riskesdas data from 2013 also stated that the cancer prevalence in Indonesia was 1.8 in every 1000 women (Riset Kesehatan Dasar, 2018). In Indonesia, cervical cancer is the second highest killer disease among women after the breast cancer. The mortality profile shows that out of the 92,000 deaths among women with cancer, the 14.1% were due to cervical cancer (World Health Organization, 2020).

In cervical cancer, the overexpression of Bcl-2 protein is known to be 14.3% in non-invasive cervical carcinoma and 65.2% in invasive cervical carcinoma (Giarnieri *et al.*, 2000). The BCL-2 protein family is an important regulator of the intrinsic pro-apoptotic and anti-apoptotic pathways. Pro-apoptosis includes protein Bax, Bak, and Bim, while anti-apoptosis consists of Bcl-2, Bcl-XL, Bcl-w, and Mcl-1 (Campbel *et al.*, 2018). Bcl-2 has an anti-apoptosis activity and is expressed in various types of cancer, especially cervical cancer (Xu *et al.*, 2018). Several studies suggest that the anti-apoptotic function of Bcl-2 is present in mitochondria as well as the endoplasmic reticulum mediated by homeostatic and dynamic effects on intracellular Ca^{2+} (Hirata *et al.*, 2012). Mitochondria are the main executor of apoptosis associated with Bcl-2 protein, which in turn cause permeabilization of the outer membrane of the mitochondria. This results in the release of cytochrome c, which then interacts with Apaf-1 that triggers the caspase-9 activation and leads to apoptosis (Wang *et al.*, 2014). The interaction of cytotoxic compounds with Bcl-2 protein occurs mainly in the BH3 domain which can cause cell death or apoptosis (Thomas *et al.*, 2013).

Currently, anticancer compounds derived from natural products are abundant because of their wide chemical diversity, physicochemical distribution, and high biological activity in inhibiting cancer growth (Newman & Giddings, 2014) (Fang *et al.*, 2018). The results of the study of gallic acid and their derivatives provide an in-silico inhibition of BRAF protein in colon cancer (Humaedi *et al.*, 2016). Furthermore, curcumin provide excellent inhibitory effects on cervical cancer cells through inhibition of telomerase activity, RAS and ERK signalling, cyclin D1, Cox-2, iNOS and mitochondrial activity (Madden *et al.*, 2009). The study of Bai *et al.*, 2015 showed that bioactive flavonoid from various natural plants, namely butein, have an inhibitory effect on migration, invasion, and induction of apoptosis in many cancer cells, especially cervical cancer (Bai *et al.*, 2015).

Mangkokan (*Polyscias scutellaria*) is known as a medicinal plant by the general

public. The components of stems, leaves, fruits, and roots have bioactive compounds that are used for the treatment of various diseases. Generally it contains a group of substances such as flavonoids, auronones, chalcones, anthocyanins and anthocyanidins (Kurniawan *et al.*, 2020). The virtual screening approach with in silico studies is one of the quick and easy steps to discover the potential of new compounds as anticancer. At this study, we identified a natural compound from the mangkokan plant (*Polyscias scutellaria*) which has potential as an inhibitor in cervical cancer by targeting the protein macromolecule Bcl-2.

MATERIALS AND METHODS

Equipment and Materials

The equipment used in this study silico molecular docking was carried out using an Intel core i5-4570 computer with 4 GB of RAM. Offline software used is MarvinSketch 15.5.11, Chimera 1.10.2, pymol 2.3.3, Rasmol 2.7.5, Ligplot 2.1 and Autodock 4.2. The materials used were the bioactive compounds of Mangkokan Plants (*Polyscias scutellaria*) and macromolecular

<https://www.rcsb.org/structure/4AQ3> with code targets 4AQ3 for BCL2.

Methods

This research was conducted at the Computing Laboratory of Binawan University. The research process consists of 5 stages as follows: arranging the target structure of the active compound derived from *Polyscias scutellaria*, researching macromolecular targets for molecular docking, preparing ligands and receptors, simulating docking between macromolecules and ligands, and analyzing docking results.

Research Stages

The preparation of the target structure of the mangkokan plant bioactive compound (*Polyscias scutellaria*) includes searching, downloading, optimization and separation of non-standard residues. The following steps macromolecular target tracking was downloaded from the RSCB Web with code 4AQ3 for BCL2. The next optimization was done using UCSF Chimera. Ligand preparation, namely the arrangement of ligands includes making 2D structures, changing the structure to 3D, and adding hydrogen atoms and Gasteiger energy. The next stage was making the Grid File Parameter (GPF) file and the Docking Parameter File (DPF) macromolecular complex with ligands used by Autodock 4.2 software. Furthermore, the docking simulation process between macromolecules and ligands which is

operated using the "common prompt" program produces a file with the format file *.glg for GPF and *.dlg to apply for DPF. The last stage is the analysis of the docking simulation results.

RESULTS AND DISCUSSION

The Molecular Characteristics of Test Ligand Compounds

In this study, the identification of bioactive compounds of mangkokan plants (*Polyscias scutellaria*), namely kaempferol, myricetin, quercetin and 3-β-[O-aG55lpha-Lrhamnopyranosyl-(1-2)-αarabionopyranosyl)oxy]-16-α hydroxyolean-12-en-28-oic acid (Johnson-Ajinwo, O. R., 2017) *et al.*, 2020). and comparison ligands (Figure 1) were in the form of molecular weight, cLog p, the number of hydrogen donors, and the number of hydrogen acceptors using the Ligandscout software. The results of the identification of characteristics are obtained in table 1 and will then be used as inhibitor compounds in in silico molecular docking studies.

Table 1. The Characteristics of Test Ligands

Compound	Molecular weight	cLogP	Number of hydrogen donors	Number of hydrogen acceptors
1 Kaempferol	286	2.46	4	6
2 Myricetin	318	1.85	6	8
3 Quercetin	302	2.16	5	7
4 3-β-[O-aG55lpha-Lrhamnopyranosyl-(1-2)-αarabionopyranosyl)oxy]-16-α hydroxyolean-12-en-28-oic acid	747	4.16	7	10
5 Topotecan (Comparison Ligands)	502	4.13	6	10

The ligands used in the in-silico molecular docking study against the target protein Bcl-2 (PDB: 4AQ3; figure 2) used the protein

database access at <https://www.rcsb.org/structure/4AQ3> were carried out by initial screening that met Lipinski's rules, namely molecular weight <500 grams/mol, number of hydrogen bonded proton donor groups <5, total hydrogen bond proton acceptor group <10, logarithmic value of the partition coefficient in water and 1-octanol <5 (Tice, 2001). Ligands that meet these rules are considered to have the potential to enter the cell membrane and be absorbed by the body.

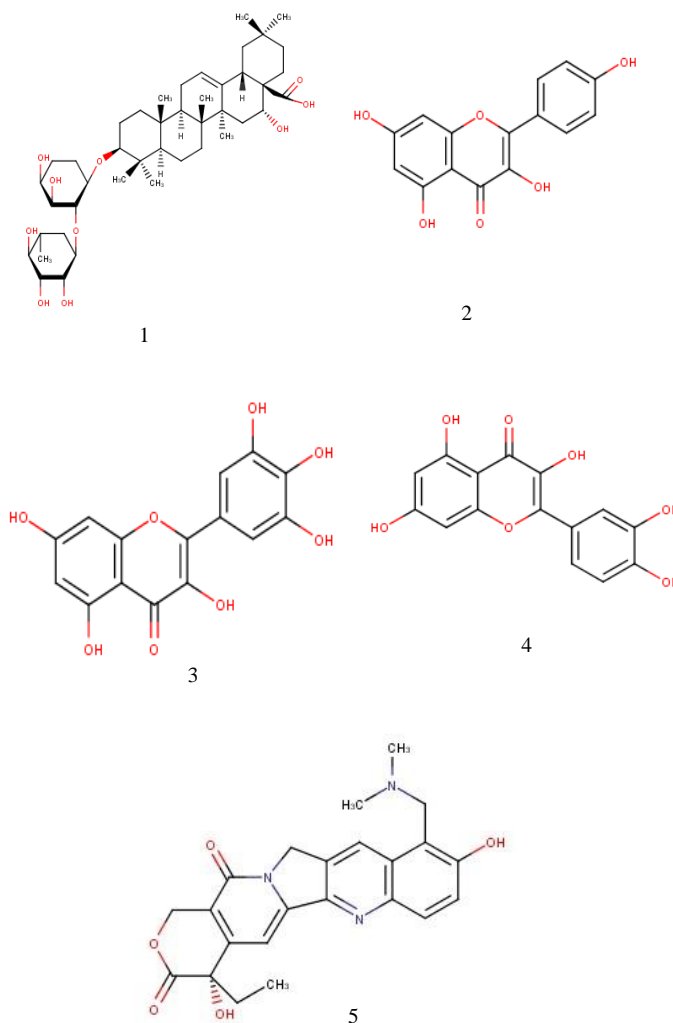


Figure 1. Structural Design of *Polyscias scutellaria* Active Compound and Its Derivates and Comparative Ligands

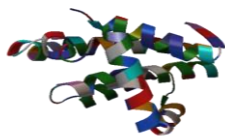


Figure 2. Structure of Bcl-2

The bioactive compounds from the *Polyscias scutellaria* plant and their derivatives used in this study have been selected according to the Lipinski rules (Table 1) so that these ligands are considered to have high bioavailability potential for the body. Bioavailability refers to the ability of a drug to be absorbed and circulated in the body (Sathishkumar *et al.*, 2012).

An Analysis and Visualization of Docking Simulation Results

In silico molecular docking studies were carried out to evaluate the effect of the ligand on Bcl-2 macromolecules. The Gibbs energy reflects the energy interactions between the ligand-protein complexes and those with the lowest energy show the more stable interactions. The docking results for bioactive compounds of mangkokan plants and comparative ligands (anticancer drugs) can be seen in Table 2.

Table 2. The Gibbs Energy(kkal/mol), and its derivatives

No	Compound Name	Gibbs Energy (ΔG) (kkal/mol)
1	3- β -[O-aG55]pha-Lrhamnopyranosyl-(1-2)- α arabionopyranosyl)oxy]-16- α hydroxyolean-12-en-28-oic acid	-6.07
2	Quercetin	-5.18
3	Myricetin	-5.43
4	Kaemferol	-6.02
5	Topotecan (Comparison Ligands)	-6.40

The docking data in table 2 shows that the mangkokan plant compounds gave varying ΔG values. Mangkokan plant compounds have the potential for inhibitory activity against cervical cancer Bcl-2 protein macromolecules as anti-proliferation and apoptosis induction with ΔG values of -6.07, -5.18, -5.43, -6.02 and -6,40 kkal/mol respectively. The mangkokan plant compound (*Polyscias scutellaria*) which provides the best inhibitory activity against the Bcl-2 protein is *Polyscias scutellaria*. In addition to ΔG , another indicator of docking results that shows the inhibitor standard between ligand-protein complexes is the inhibition constant (K_i), the number of hydrogen bonds and electrostatic energy can be seen in Table 3.

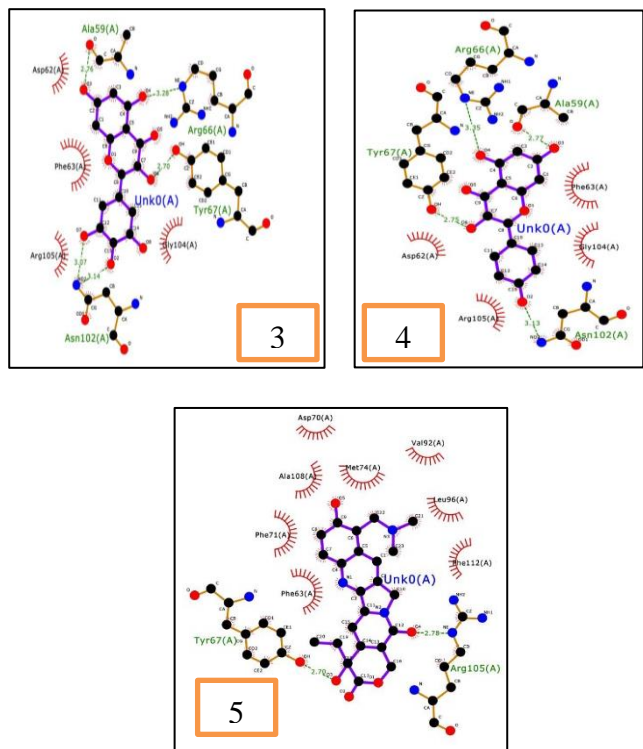
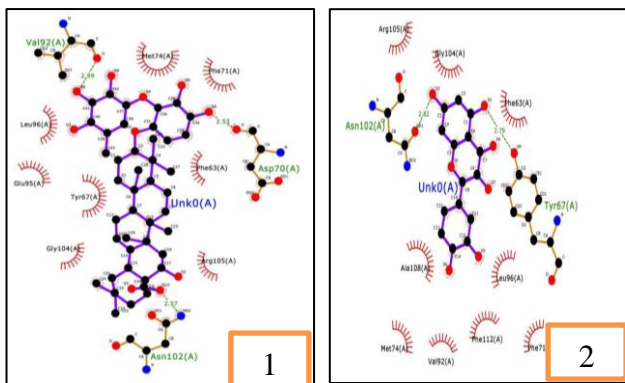
Table 3. Simulation results of docking test ligands and comparison ligands at the Androgen receptor

No	Compound name	K_i (μM)	$\sum H $	Electrostatic Energy
1	3- β -[O-aG55]pha-Lrhamnopyranosyl-(1-2)- α arabionopyranosyl)oxy]-16- α hydroxyolean-12-en-28-oic acid	35.38	3	-0.46
2	Quercetin	160.12	3	-0.008
3	Myricetin	104.31	2	-0.06
4	Kaemferol	38.98	2	-0.12
5	Topotecan (Comparison Ligand)	38.80	0	0.90

Inhibition constant is an important aspect that must be considered in ligand and receptor interactions because it is related to binding affinity. A lower affinity value indicates that a compound requires less energy to interact with the receptor. Thus, a lower binding affinity value has a greater potential to be able to interact with the target protein (Pangastuti *et al.*, 2016)

In silico molecular docking studies have a good indicator when comparing the Gibbs energy value (ΔG), inhibition constant, and the number of hydrogen interactions as a standard inhibitor. Strong complex bonds are formed with a low Gibbs energy value (ΔG), an inhibition constant and a large number of hydrogen interactions. As seen in tables 1 and 2, according to the criteria of a good inhibitor, 3- β -[O-aG55]pha-Lrhamnopyranosyl-(1-2)- α arabionopyranosyl]oxy]-16- α hydroxyolean-12-en-28-oic acid(1) and kaempferol with Gibbs energy (ΔG), inhibition constant, number of interactions, and hydrogen electrostatic energy for 3- β -[O-aG55]pha-Lrhamnopyranosyl-(1-2)- α arabionopyranosyl]oxy]-16- α hydroxyolean-12-en-28-oic acid and kaempferol in table 3 are -6.01 kkal/mol, 35.38, 3, and -0.46 respectively. Topotecan compounds as comparison ligands provide a strong activity with Gibbs energy (ΔG), inhibition constant, number of interactions, and hydrogen electrostatic energy being 38.80, 0, and 0.90.

Of the four test ligands, the bioactive compound 3- β -[O-aG55]pha-Lrhamnopyranosyl-(1-2)- α arabionopyranosyl]oxy]-16- α hydroxyolean-12-en-28-oic acid, has better potential as an inhibitor against the target protein Bcl-2 in cervical cancer with a value of ΔG -6.07 kkal/mol, Ki 35.38 μ M and electrostatic energy of -0.46 whereas compared to the comparator ligands the value of ΔG was slightly larger, it is likely that this is due to the number of alkyl groups, OH, and benzene which affect the binding affinity.



Figures 3. Intercation of *Polyscias scutellaria* ligands and their derivatives (1. 3- β -[O-aG55]pha-Lrhamnopyranosyl-(1-2)- α arabionopyranosyl]oxy]-16- α hydroxyolean-12-en-28-oic acid, 2. Quarecetin, 3. Mircyctin, 4. Kaemferol and comaprison ligands (5. Topotecan) with Bcl-2 macromolecules

Figure 3 shows that the interaction of the ligand with the Bcl-2 macromolecule is bound to certain amino acids. Ligand 1 is bound to amino acids ASP70, VAL92 and ASN102; ligand 2 is bound to the amino acids TYR67 and ASN102; ligand 3 is bound to the amino acids ALA59, ARG66, TYR67 and ASN102; ligand 4 is bound to the amino acids ALA59, ARG66, TYR67 and ASN102; and ligand 5 is bound to the amino acids TYR67 and ARG105. In this interaction a strong enough hydrogen bond is formed with an average distance of $<3.5\text{\AA}$ with the possibility of inhibition or apoptosis in cancer cells through binding to the BH3 domain. Research conducted by Sathiskumar et al (2012) showed a strong enough interaction between the ligands of (ginsenosides) natural compounds against Bcl-2 protein, namely the amino acids TYR67, GLU95, ARG142, ARG12, THR137, and ASN131. In addition, the natural compounds of the chalcone group provide a strong binding to

the Bcl-2 protein in the amino acids ALA146 and ARG143 (Chen *et al.*,2017). There is a correlation, between chalcone and flavonoids which are secondary metabolites that have potential as anti-cancer properties. Using the same macromolecule, namely BCL-2, the mechanism of action is substantially the same.

CONCLUSION

The results of the virtual screening approach through in silico studies have shown that the bioactive compounds of mangkokan (*Polyscias scutellaria*), namely kaemferol, myrcetin, quarcetin and 3-β-[O-αG55]pha-Lrhamnopyranosyl-(1-2)-arabionopyranosyl(oxy)-16- α hydroxyolean-12-en-28-oic acid is the most potential compound as candidates for cervical cancer drugs. Based on three important indicators that ΔG -6.07 kkal/mol, Ki 35.38 μM and ΣIH -0.46. Virtual screening simulated the first step in the development of new drug candidate searches. Further research needs to be done in vitro to determine the potential for inhibition of cervical cancer cell lines.

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