Potency of *Cyperus rotundus* bioactive compound against anti-apoptotic protein: an in silico approach

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Abstract. The study aimed to analyze the potency of *Cyperus rotundus* bioactive compounds to inhibit the anti-apoptotic protein Bcl-2 and Bcl-xl by in silico approach. Ten bioactive compounds were used in this study, such as apigenin, aureusidin, cyperol, cyperusol A1, cyperusol B2, cyperusol D, luteolin, methyltartonic, quercetin, and scaberin. The 3D structure of ligands and protein was retrieved from PubChem and Protein Data Bank (www.rcsb.org). The molecular docking analysis was done by AutoDock Vina in PyRx v.0.8. The results showed that the lowest binding affinity against bcl-2 was obatoclax as control ligand and followed by scaberin, aureusidin, luteolin, apigenin, and quercetin with binding affinity score -7.4, -7, -6.9, -6.9, and -6.8 kcal/mol, respectively. Those ligands also found have the best binding affinity against Bcl-xl where apigenin, luteolin, and quercetin were -8 kcal/mol and lower than the binding affinity of obatoclax, aureusidin, and scaberin (-7.8, -7.8, and -7.3 kcal/mol, respectively). Based on the prediction of cytotoxic potential of drug-like compounds using Pass program showed the best cytotoxic activity of obatoclax against HT-29 cell line (pa>0.6), apigenin against Hs 683 (pa>0.5), luteolin against Hs 683 (pa>0.5), and quercetin against CWR22R (pa>0.5). In conclusion, the bioactive compounds of *Cyperus rotundus* exhibited a potential anti-cancer activity through the inhibition of Bcl-2 and Bcl-xl. Further study needs to justify the anti-cancer mechanism of *Cyperus rotundus* extract.

1. Introduction

Cancer is one of the leading causes of death and became one of the main health issues worldwide. Based on the latest global cancer data of the Globocan Data [1], the case of cancer elevates to 18.1 million and 9.6 million cancer deaths in 2018. Biological process dysregulation including cell division and cell death affects healthy cells and homeostasis cause to formation and development of cancer. The elevation of the cell division pathway caused by the degradation of the apoptotic pathway occurs during cancer progression and becomes these disease hallmarks [2].

The most common mechanism in the elimination of undesirable defected, and dangerous cells or that undergo uncontrolled cellular proliferation is apoptosis. The Protein Bcl-2 family plays a vital role in apoptosis regulation and implementation. This protein is commonly located in the outer membrane of mitochondria. There are two classes of protein Bcl-2 family, one involved in the promotion (pro-apoptotic) and another involved in the inhibition (anti-apoptotic) of apoptosis [3]. Interaction of Bcl-2
family protein with activators such as tBid, Puma, and Bind occur during apoptosis. These activators then release from anti-apoptotic protein (i.e. Bcl-2, Bcl-w, Bcl-XL, Mcl-1, and A1) caused by effector protein such as Bad, Noxa, Bik, Hrk, Bnip3, and Bmf, which then initiate permeabilization of the mitochondrial outer membrane (MOMP). Cytochrome c and apoptosis-inducing factor (AIF) then release and lead to the initiation of apoptotic machinery [4]. Inhibition of anti-apoptotic protein becomes one of target cancer therapy and drug discovery.

Herbal medicine is known as a promising future drug in disease and health management. Bioactive compounds contained in the plant have an essential role in the development of drug discovery [5]. *Cyperus rotundus* is a traditional medicine that has been widely used in Asia, Africa, and Europe, and showed a potency as an anticancer agent. *Cyperus rotundus*, also known as nutgrass grows in tropical, subtropical, and temperate regions. *Cyperus rotundus* extract can regulate apoptotic gene expression in human cervical carcinoma (HeLa) cell lines in vitro [6]. The antioxidant and anti-proliferative capacity of *Cyperus rotundus* extract inhibits xanthine oxidase (XO) enzyme, lipid peroxidase, and pressing apoptosis in the erythroleukemia cell line, K562 [7]. Methanolic extract of *Cyperus rotundus* is known able to induce apoptosis in various cell lines, including MCF-7. HeLa, HEP-G2, PC-3, and HT-29 [8]. Ethanolic extract of its plant also reported can inhibit cell proliferation in MDA-MB-231 and MDA-MB-468 (triple-negative breast cancer cell line) that linked to cell cycle arrest in G0/G1 phase, induce apoptosis via BAX protein expression promotion, and inhibit the expression of Bcl-2 family [9]. The study aimed to analyze the potency of *Cyperus rotundus* bioactive compounds to inhibit the anti-apoptotic protein Bcl-2 and Bcl-xl by in silico approach.

2. Methods

2.1. Data preparation

Bioactive compounds of *Cyperus rotundus* used in this study were (1) apigenin, (2) aureusidin, (3) cyperol, (4) cyperol A1, (5) cyperosol B2, (6) cyperosol D, (7) luteolin, (8) methyltartronic, (9) quercetin, and (10) scaberin. The control ligand used in this study was obatoclax. Obatoclax is a pre-clinical Bcl-2 inhibitor that acts as the antagonist of anti-apoptotic protein [10]. The 3D molecular structure of bioactive compound or ligand were retrieved from PubChem chemical databases which were then converted to .pdb file format by using Discovery Studio Visualizer 4.0 software.

The structures of Bcl-2 and Bcl-xl protein were retrieved from RCSB Protein Data Bank and saved as .pdb. That protein was prepared by deleting the water molecule using a discovery studio and then saved for further analysis.

2.2. Docking procedure

Molecular docking simulation was done by using the AutoDock Vina program in PyRx 0.8 software. Ligands and target structures were docked via blind docking, which means docking was done randomly or not specifically at the active site. The maximum grid box value was set. The docking runs were completed for each ligand. The binding affinity of the interaction was observed, and the file was saved and visualized by using Discovery Studio to analyze the amino acid residues binding site for each ligand. Visualization of 2D and 3D interaction was obtained from Discovery Studio.

2.3. PASS CLC-Pred analysis

Prediction of cytotoxic activity on various cell lines of selected bioactive compounds of *Cyperus rotundus* was analyzed using PASS CLC-Pred Program. The average accuracy of PASS prediction was 95% for about 7000 kinds of biological activity analyze through the relationship of structure-activity. Cytotoxic activity was analyzed through the interaction of drug-like compounds with tumor and non-tumor cell lines. The data input into the PASS program is presented as SMILES and MOL or SDF file. There would two estimated probabilities as output: Pa (to be ‘active’) and Pi (to be ‘inactive’). If data obtained from PASS analysis Pa>Pi showed and considered as an active compound. The stronger Pa (Pa>30%), the increased chance on the confirmation experimental of the predicted activities [11].
3. Results and Discussion

3.1. Molecular Docking

Bcl-2 and Bcl-xl play an important role in the intrinsic pathway of apoptosis. These two proteins are involved in the mechanism of survival and anti-apoptotic. Four α-helical Bcl-2 they share as homology domain (BH) BH1-4, and apoptosis can occur when Bax and Bak are sharing homology in BH1-3 [12]. Unfortunately, overexpression of Bcl-2 and Bcl-xl anti-apoptotic protein is well found in cancer, so it needs to be blocked to balance the shifts towards multi-domain pro-apoptotic proteins Bax and Bak.

Molecular docking was done, and the interaction of ligands and proteins was observed. The interaction of bioactive compounds and anti-apoptotic proteins were analyzed and ranked based on the binding affinity. This study found that obatoclax showed the lowest binding affinity against bcl-2 protein (-6.8 kcal/mol), followed by scaberin (-7 kcal/mol), aureusidin (-6.9 kcal/mol), luteolin (-6.9 kcal/mol), apigenin (-6.8 kcal/mol), and quercetin (-6.8 kcal/mol). Furthermore, the analysis of bioactive compounds binding affinity against Bcl-xl protein showed that apigenin, luteolin, and quercetin demonstrated lower binding affinity (-8 kcal/mol) comparable to obatoclax (-7.8 kcal/mol). The summary of binding affinity (kcal/mol) analysis of this study showed in Table 1.

The observation of binding affinity score was done as the primary function as force-field based. The lower the binding affinity score, the less intermolecular force between ligand-protein, and the more stable the interaction of the complex formed [13].

| Ligand (PubChem ID) | Structure | Bcl-2 (1GJH) | Bcl-xl (1MAZ) |
|---------------------|-----------|--------------|---------------|
| Apigenin (5280443)  | ![Apigenin Image](image1.jpg) | -6.8          | -8            |
| Aureusidin (5281220)| ![Aureusidin Image](image2.jpg) | -6.9          | -7.8          |
| Cyperol (14076601)  | ![Cyperol Image](image3.jpg)  | -6.3          | -6.2          |
Cyperusol A1 (10083471)  
-5.8  -6.9

Cyperusol B2 (11310916)  
-6  -5.9

Cyperusol D (11311311)  
-5.7  -6

Luteolin (5280445)  
-6.9  -8

Methyltartronic (136386)  
-4.1  -5.1
3.2. Binding Interaction with Bcl-2

The interaction and amino acid residues involvement of investigated bioactive compounds against Bcl-2 protein were summarized in Table 2. Results showed the interaction of obatoclax and Bcl-2 protein consist of a hydrogen bond, an electrostatic, and three hydrophobic bonds. However, a hydrogen bond was formed involving amino acid residue Tyr9 as a conventional hydrogen bond. In contrast, the three hydrophobic interactions were formed involving hydrophobic amino acids His186 and Tyr196 as Pi-Sigma, Pi-Pi T-shaped, Pi-Alkyl interactions (Figure 1a, b). Apigenin showed more hydrogen bonds than obatoclax involving Asn11 amino acid residue. One electrostatic bond and two hydrophobic bonds are involving Tyr9 and His186 amino acid residues as Pi-Pi T-Shaped interaction (Fig 1c, d). Aureusidin interaction with Bcl-2 showed more hydrogen bond in amino acid residues Gly193, Asp10, and Trp195 as conventional hydrogen bond and Pi-Anion interactions. Its interaction also found three hydrophobic interactions involving hydrophobic amino acid residues His186 and Ala4, and they were Pi-Pi T-shaped and Pi-Alkyl interaction (Fig 1e, f). Three hydrogen bonds are found in the interaction of luteolin towards Bcl-2 protein, and it's the only interaction formed by this complex. Amino acid residues involved in this complex were Thr41, Asn39, and Arg6 as conventional hydrogen bond interactions (Fig 1g, h). Besides that, apigenin showed three hydrogens and three hydrophobic bonds as its interaction with Bcl-2 protein. The three hydrogen bonds formed involving amino acid residues Tyr9, His186, and Ala4 as conventional and carbon-hydrogen bond interactions (Fig 1i, j). Scaberin and Bcl-2 interaction formed three hydrogen bonds and two hydrophobic bonds. Amino acid residues Asp196, Ala4, and Gly194 were found in the three hydrogen bonds as carbon-hydrogen bond interactions. Two hydrophobic bonds were found involving amino acid residue Tyr9 as Pi-Pi T-Shaped and Pi-Alkyl interaction (Figure 1k, l).

Results showed the investigated bioactive compounds of *Cyperus rotundus* demonstrated more hydrogen and hydrophobic bonds comparing to obatoclax. The hydrogen bond is fundamental and becoming the main reason for the selectivity of the ligand-protein complex. Docking and scoring were mostly done by observing the detailed analysis of binding interaction with hydrogen bond based [14].

| Compound         | Hydrogen Bond | Hydrophobic Bond |
|------------------|---------------|-----------------|
| Quercetin (5280343) | -6.8          | -8              |
| Scaberin (42608130)   | -7            | -7.3            |
| Obatoclax (16681698)   | -7.4          | -7.8            |
Some amino acid residues involved in the interaction of obatoclax towards Bcl-2 were the same as in complex bioactive compounds against Bcl-2 protein. It showed the same amino acid residues involved had a relatively significant role in the inhibition of Bcl-2 antiapoptotic protein.

Table 2. Interaction and amino acid residues involvement of investigated bioactive compounds against Bcl-2 protein.

| Ligand | Name | From Chemistry | To Chemistry | Distance | Category | Types of |
|--------|------|----------------|--------------|----------|----------|----------|
| Obatoclax | A: ASP196: OD2 - :LIG1 | Negative :LIG1:Pi-Orbitals | :LIG1:Pi-Orbitals | 3.7311 | Electrostatic | Pi-Anion |
| A: TYR9 : H | H-Donor | A: TYR9:OH | H-Acceptor | 2.81199 | Hydrogen Bond | Convention Hydrogen Bond |
| A: HIS186 | H | A: TYR9: Pi-Orbitals | A: TYR9: Pi-Orbitals | 5.3284 | Hydrophobic | Pi-Pi T-shaped |
| A: TYR9: C | H-Donor | :LIG1:C Alkyl | :LIG1:C Pi-Orbitals | 5.07365 | Hydrophobic | Pi-Alkyl |
| Apigenin | A: ASN11: HN - :LIG1:O | H-Donor | :LIG1:O H-Acceptor | 2.0239 | Hydrogen Bond | Convention Hydrogen Bond |
| A: ASP10: OD1 - :LIG1 | Negative :LIG1:Pi-Orbitals | :LIG1:Pi-Orbitals | 4.39435 | Electrostatic | Pi-Anion |
| A: TYR9 : H | H-Donor | A: TYR9:OH | H-Acceptor | 2.16599 | Hydrogen Bond | Convention Hydrogen Bond |
| :LIG1: H - :A:GLY193 | O | H-Donor | A:GLY193:O H-Acceptor | 2.16599 | Hydrogen Bond | Convention Hydrogen Bond |
| Auresidin | A: TRP195: HE1 - :LIG1:H | H-Donor | :LIG1:O H-Acceptor | 2.30582 | Hydrogen Bond | Convention Hydrogen Bond |
| A: ASP10: OD1 | Negative :LIG1:Pi-Orbitals | :LIG1:Pi-Orbitals | 2.57195 | Hydrophobic | Pi-Pi T-shaped |
| A: HIS186 | H | A: ASP10: OD1 | A: ASP10: OD1:Pi-Orbitals | 2.57195 | Hydrogen Bond | Convention Hydrogen Bond |
| A: HIS186 | H | H-Donor | :LIG1:Pi-Orbitals | 4.4462 | Hydrophobic | Pi-Pi T-shaped |
| A: HIS186 | H | H-Donor | :LIG1:Pi-Orbitals | 4.96561 | Hydrophobic | Pi-Pi T-shaped |
| Ligand | Orbits 1 | Orbits 2 | ic | shaped |
|--------|----------|----------|----|--------|
| LIG1 - A:ALA4 | Pi-Orbitals | A:ALA4 | 4.60055 | Hydrophobic Pi-Alkyl |
| LIG1:H - A:THR41:OG1 | H-Donor | A:THR41:OG1 | 2.1659 | Hydrogen Bond Convention Hydrogen Bond |
| LIG1:H - A:ASN39:OD1 | H-Donor | A:ASN39:OD1 | 2.53807 | Hydrogen Bond Convention Hydrogen Bond |
| LIG1:H - A:ARG6:O | H-Donor | A:ARG6:O | 2.82186 | Hydrogen Bond Convention Hydrogen Bond |
| LIG1:H - A:TYR9:O | H-Donor | A:TYR9:O | 2.29589 | Hydrogen Bond Convention Hydrogen Bond |
| LIG1:H - A:TYR9:O | H-Donor | A:TYR9:O | 3.00767 | Hydrogen Bond Convention Hydrogen Bond |
| LIG1:H - A:TYR9:O | H-Donor | A:TYR9:O | 2.46497 | Hydrogen Bond Carbon Hydrogen Bond |
| LIG1:H - A:TYR9:O | H-Donor | A:TYR9:O | 2.80409 | Hydrophobic Pi-Sigma |
| LIG1:H - A:TYR9:O | H-Donor | A:TYR9:O | 5.25734 | Hydrophobic Pi-Pi T-shaped |
| LIG1:H - A:TYR9:O | H-Donor | A:TYR9:O | 4.87583 | Hydrophobic Pi-Alkyl |
| LIG1:C - A:ASP196:OD1 | H-Donor | A:ASP196:OD1 | 3.62298 | Hydrogen Bond Carbon Hydrogen Bond |
| LIG1:H - A:TYR9:O | H-Donor | A:TYR9:O | 2.56866 | Hydrogen Bond Carbon Hydrogen Bond |
| LIG1:H - A:TYR9:O | H-Donor | A:TYR9:O | 2.95621 | Hydrogen Bond Carbon Hydrogen Bond |
| LIG1:H - A:TYR9:O | H-Donor | A:TYR9:O | 5.48296 | Hydrophobic Pi-Pi T-shaped |
| LIG1:H - A:TYR9:O | H-Donor | A:TYR9:O | 4.8967 | Hydrophobic Pi-Alkyl |

**Quercetin**

**Scaberin**
Figure 1. Interaction of investigated bioactive compounds toward Bcl-2 protein, a-b. Obatoclax, c-d. Apigenin, e-f. Aureusidin, g-h. Luteolin, i-j. Quercetin, k-l. Scaberin and Bcl-2 complex.
3.3. Binding Interaction with Bcl-xl

Investigated bioactive compounds and Bcl-xl protein was summarized in Table 3. This study found that apigenin, luteolin, and quercetin had a lower binding affinity toward Bcl-xl compared to obatoclax. Apigenin interaction with Bcl-xl consists of a hydrogen bond and nine hydrophobic bonds. Amino acid residue phe05 was involved in hydrogen bond as conventional hydrogen bond interaction. Hydrophobic interaction formed involving amino acid residues Tyr101, Phe105, Phe97, Arg103, Arg139, Ala142 as Pi-Pi stacked, Pi-Pi T-Shaped, and Pi-alkyl interaction (Figure 1a, b). Aureusidin and Bcl-xl interaction consist of four hydrogen bonds, and nine hydrophobic interactions involved amino acid residues Tyr101, Arg103, Ala104, Phe105, Phe97, Arg139, and Ala142. The type of interaction formed includes conventional hydrogen bond, Pi-Pi Stacked, Pi-Pi T-Shaped, and Pi-Alkyl (Figure 1c, d). The three hydrogen bonds and nine hydrophobic interactions in the complex of luteolin and Bcl-xl involved amino acid residues Tyr101, Arg103, Phe105, Phe97, Arg139, and Ala142 as conventional hydrogen bond, Pi-Pi stacked, Pi-Pi T-shaped, and Pi-Alkyl (Figure 1e, f). A similar interaction was also demonstrated on quercetin against Bcl-xl. Three hydrogen bonds and nine hydrophobic interactions involving similar amino acid residues and type of interaction as luteolin (Figure 1g, h). Scaberin showed for hydrogen bond and five hydrophobic interactions. The amino acid residues involved in the interaction include Asn136, Arg139, Leu130, Leu108, Phe97, Tyr101, and Phe105. The type of interaction was a conventional hydrogen bond, Pi-Donor Hydrogen Bond, Alkyl, and Pi alkyl (Figure 1i, j).

Besides the fundamental function of the hydrogen bond, hydrophobic interaction also plays a vital role in the complex ligand-protein interaction. The high amount of hydrophobic interactions were considered for the optimization of its complex that is required to enhance the molecular weight of ligand, lipophilicity, and ADMET properties of ligands [15]. A high amount of hydrogen bonds and hydrophobic interactions in our bioactive compound against anti-apoptotic protein demonstrated a proportional and suitable complex ligand-protein as an anticancer drug candidate.

Table 2. Interaction and amino acid residues involvement of investigated bioactive compounds against Bcl-xl protein.

| Ligand | Name | From Chemistry | To | To Chemistry | Distance | Category | Types of | Types of |
|--------|------|----------------|----|--------------|----------|----------|----------|----------|
|        |      | H-Donor        | :LIG1:H | H-Donor      | 2.88582  | Hydrogen Bond | Conventional Hydrogen Bond |        |
|        |      | A:GLU98:OE1    | A:GLU98:OE1 | H-Donor | 3.23756 | Hydrogen Bond | Conventional Hydrogen Bond |        |
| Obatoclax | A:ARG10:2:NE | :LIG1:O | :LIG1:O | H-Donor | 3.81901 | Electrostatic | Pi-Anion |        |
|        |      | Negative        | :LIG1 | Pi-Orbitals | 3.87812 | Hydrophobic |Pi-Sigma |        |
|        |      | :LIG1:C         | :LIG1:C:CB | C-H | 4.34734 | Hydrophobic | Alkyl |        |
|        |      | Alkyl           | A:ALA104 | Alkyl | 4.4564 | Hydrophobic | Pi-Alkyl |        |
|        |      | :LIG1 - A:ALA149 | Pi-Orbitals | A:ALA149 | 4.8863 | Hydrophobic | Pi-Alkyl |        |
|        |      | Pi-Orbitals    | A:LEU99 | Alkyl |        | | |        |
| Complex     | Atom 1  | Atom 2  | Property       | Value    | Description          |
|-------------|---------|---------|----------------|----------|----------------------|
| A:PHE10     | Pi-Orbitals | LIG1:C | Alkyl          | 4.60788  | Hydrophobic          |
| A:PHE10     | H-Donor | LIG1:C  | H-Acceptor     | 3.23251  | Hydrogen Bond        |
| :LIG1      | Pi-Orbitals | A:TYR101 | H-Acceptor     | 5.80815  | Hydrophobic          |
| :LIG1      | Pi-Orbitals | A:TYR101 | H-Acceptor     | 5.12832  | Hydrophobic          |
| :LIG1      | Pi-Orbitals | A:TYR101 | H-Acceptor     | 4.12915  | Hydrophobic          |
| :LIG1      | Pi-Orbitals | A:TYR101 | H-Acceptor     | 5.21893  | Hydrophobic          |
| :LIG1      | Pi-Orbitals | A:TYR101 | H-Acceptor     | 5.15479  | Hydrophobic          |
| :LIG1      | Pi-Orbitals | A:ARG103 | Alkyl          | 5.38519  | Hydrophobic          |
| :LIG1      | Pi-Orbitals | A:ARG139 | Alkyl          | 5.25346  | Hydrophobic          |
| :LIG1      | Pi-Orbitals | A:ALA142 | Alkyl          | 4.13728  | Hydrophobic          |
| LIG1:H     | H-Donor | A:TYR101:OH | H-Acceptor | 1.89535  | Hydrogen Bond        |
| LIG1:H     | H-Donor | A:TYR101:O  | H-Acceptor    | 2.57069  | Hydrogen Bond        |
| A:ARG10    | H-Donor | LIG1:O    | H-Acceptor    | 3.32441  | Hydrogen Bond        |
| A:ALA10    | H-Donor | LIG1:O    | H-Acceptor    | 2.87262  | Hydrogen Bond        |
| LIG1       | Pi-Orbitals | A:TYR101 | H-Acceptor    | 4.99899  | Hydrophobic          |

Apigenin

Aureusidin
| Interaction          | Reference Residue | Reference Atom  | Ligand Atom   | Distance (Å) | Hydrophobic Interaction | Hydrogen Bond Type | Notes     |
|----------------------|-------------------|-----------------|---------------|--------------|------------------------|--------------------|----------|
| :LIG1 - A:TYR10      | Pi-Orbitals       | A:TYR101        | Pi-Orbitals   | 5.6536       | Hydrophobic            | Pi-Pi Stacked      |          |
| :LIG1 - A:PHE10      | Pi-Orbitals       | A:PHE105        | Pi-Orbitals   | 4.83973      | Hydrophobic            | Pi-Pi Stacked      |          |
| :LIG1 - A:PHE97      | Pi-Orbitals       | A:PHE97         | Pi-Orbitals   | 5.51018      | Hydrophobic            | Pi-Pi T-shaped     |          |
| :LIG1 - A:TYR10      | Pi-Orbitals       | A:TYR101        | Pi-Orbitals   | 5.2656       | Hydrophobic            | Pi-Pi T-shaped     |          |
| :LIG1 - A:ARG10      | Pi-Orbitals       | A:ARG103        | Alkyl         | 5.25185      | Hydrophobic            | Pi-Alkyl           |          |
| :LIG1 - A:ARG13      | Pi-Orbitals       | A:ARG139        | Alkyl         | 5.22713      | Hydrophobic            | Pi-Alkyl           |          |
| :LIG1 - A:ALA14      | Pi-Orbitals       | A:ALA142        | Alkyl         | 4.37576      | Hydrophobic            | Pi-Alkyl           |          |
| :LIG1 - A:TYR10      | H-Donor           | :LIG1:O         | H-Acceptor    | 2.90129      | Hydrogen Bond          | Conventional       |          |
| :LIG1 - A:TYR10      | H-Donor           | :LIG1:O         | H-Acceptor    | 3.24085      | Hydrogen Bond          | Conventional       |          |
| :LIG1 - A:TYR10      | H-Donor           | :LIG1:O         | H-Acceptor    | 3.25387      | Hydrogen Bond          | Conventional       |          |
| :LIG1 - A:TYR10      | Pi-Orbitals       | A:TYR101        | Pi-Orbitals   | 5.81435      | Hydrophobic            | Pi-Pi Stacked      |          |
| :LIG1 - A:PHE10      | Pi-Orbitals       | A:PHE105        | Pi-Orbitals   | 5.1763       | Hydrophobic            | Pi-Pi Stacked      |          |
| :LIG1 - A:PHE10      | Pi-Orbitals       | A:PHE105        | Pi-Orbitals   | 4.13758      | Hydrophobic            | Pi-Pi Stacked      |          |
| :LIG1 - A:PHE97      | Pi-Orbitals       | A:PHE97         | Pi-Orbitals   | 5.29573      | Hydrophobic            | Pi-Pi T-shaped     |          |
| :LIG1 - A:TYR10      | Pi-Orbitals       | A:TYR101        | Pi-Orbitals   | 5.23715      | Hydrophobic            | Pi-Pi T-shaped     |          |

**Luteolin**

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| Interaction | Donor  | Acceptor | Interaction Type | Distance (Å) | Category         |
|-------------|--------|----------|------------------|--------------|------------------|
| :LIG1 - A:ARG103 | Alkyl  | :LIG1:O  | H-Donor          | 5.38376      | Hydrophobic      |
| :LIG1 - A:ARG139  | Alkyl  | :LIG1:O  | H-Donor          | 5.19628      | Hydrophobic      |
| :LIG1 - A:ALA142  | Alkyl  | :LIG1:O  | H-Donor          | 4.09603      | Hydrophobic      |
| A:TYR10 - 1:OH   | H-Donor | :LIG1:O  | H-Acceptor       | 2.84878      | Hydrogen Bond    |
| A:ARG103 - 3:N    | H-Donor | :LIG1:O  | H-Acceptor       | 3.3054       | Hydrogen Bond    |
| A:ARG103 - 3:NE   | H-Donor | :LIG1:O  | H-Acceptor       | 3.0658       | Hydrogen Bond    |
| :LIG1 - A:TYR101  | Pi-Orbitals | A:TYR101 | Pi-Orbitals      | 5.81552      | Hydrophobic      |
| :LIG1 - A:TYR101  | Pi-Orbitals | A:TYR101 | Pi-Orbitals      | 5.19705      | Hydrophobic      |
| :LIG1 - A:PHE105  | Pi-Orbitals | A:PHE105 | Pi-Orbitals      | 4.11686      | Hydrophobic      |
| :LIG1 - A:PHE105  | Pi-Orbitals | A:PHE105 | Pi-Orbitals      | 4.94474      | Hydrophobic      |
| :LIG1 - A:PHE97   | Pi-Orbitals | A:PHE97  | Pi-Orbitals      | 5.28333      | Hydrophobic      |
| :LIG1 - A:TYR101  | Pi-Orbitals | A:TYR101 | Pi-Orbitals      | 5.22909      | Hydrophobic      |
| :LIG1 - A:ARG103  | Alkyl  | :LIG1:O  | H-Donor          | 5.41142      | Hydrophobic      |
| :LIG1 - A:ARG139  | Alkyl  | :LIG1:O  | H-Donor          | 5.22121      | Hydrophobic      |
| :LIG1 - A:ALA142  | Alkyl  | :LIG1:O  | H-Donor          | 4.12804      | Hydrophobic      |
| A:ASN13 - 6:ND2   | H-Donor | :LIG1:O  | H-Acceptor       | 3.14195      | Hydrogen Bond    |
| A:ARG13 -           | H-Donor | :LIG1:O  | H-Acceptor       | 3.05099      | Hydrogen Bond    |

**Quercetin**

**Scaberin**
| Bond  | al Hydrogen Bond |
|-------|------------------|
| A:LEU13 9:NH2 - :LIG1:O | H-Donor :LIG1:O H-Acceptor 3.48011 Hydrogen Bond |
| A:ARG13 9:NE - :LIG1 | H-Donor :LIG1 Pi-Orbitals 3.82355 Hydrogen Bond Pi-Donor Hydrogen Bond |
| :LIG1:C - A:LEU10 8 | Alkyl A:LEU108 Alkyl 4.05996 Hydrophobic Alkyl |
| :LIG1 - A:ARG13 9 | Pi-Orbitals A:ARG139 Alkyl 4.03026 Hydrophobic Pi-Alkyl |
| A:PHE97 - :LIG1:C | Pi-Orbitals :LIG1:C Alkyl 4.76873 Hydrophobic Pi-Alkyl |
| A:TYR10 1 - :LIG1:C | Pi-Orbitals :LIG1:C Alkyl 3.82053 Hydrophobic Pi-Alkyl |
| A:PHE10 5 - :LIG1:C | Pi-Orbitals :LIG1:C Alkyl 4.92118 Hydrophobic Pi-Alkyl |

![Diagram a](image-a)

![Diagram b](image-b)

![Diagram c](image-c)

![Diagram d](image-d)
Figure 2. Interaction of investigated bioactive compounds toward bcl-xl protein, a-b. apigenin, c-d. aureusidin, e-f. luteolin, g-h. quercetin, i-j. scaberin and bcl-2 complex.

3.4. Cytotoxicity Analysis
The cytotoxicity analysis of investigated bioactive compounds analyzed using PASS CLC-Pred Program. The increase Pa value of the threshold would reduce the number of false-positive prediction [16]. Pa>0.4 was used in this study. The compounds demonstrated cytotoxicity activity on various cell lines with different Pa scores. Apigenin and luteolin showed top predicted cytotoxic effect on oligodendroglioma, gastric carcinoma, and small cell lung carcinoma cell line. Aureusidin showed its best cytotoxic effect only on the colon adenocarcinoma cell line. Quercetin demonstrated its top cytotoxic activity on prostate carcinoma epithelial cell line, oligodendroglioma, non-small cell lung carcinoma, and small cell lung carcinoma cell line. Whereas scaberin only showed its top predicted cytotoxic effect on small cell lung carcinoma and breast carcinoma cell line. The use of PASS program prediction as a way to have a rapid prediction of potentially bioactive compounds in various biological activity, including the anticancer mechanism. It gives possible information about the preliminary study on the toxicity activity of active compounds [17].
Table 4. Apigenin cytotoxicity analysis using PASS CLC-Pred

| Bioactive compound | Pa    | Pi     | Cell-line | Cell-line full name | Tissue | Tumor type |
|--------------------|-------|--------|-----------|---------------------|--------|------------|
| Apigenin           | 0.587 | 0.029  | Hs 683    | Oligodendrogloma    | Brain  | Glioma     |
|                    | 0.474 | 0.014  | HOP-18    | Non-small cell lung carcinoma | Lung    | Carcinoma  |
| Aureusidin         | 0.409 | 0.005  | SGC-7901  | Gastric carcinoma   | Stomach | Carcinoma  |
|                    | 0.440 | 0.039  | NCI-H187  | Small cell lung carcinoma | Lung    | Carcinoma  |
| Luteolin           | 0.523 | 0.049  | Hs 683    | Oligodendrogloma    | Brain  | Glioma     |
|                    | 0.456 | 0.017  | HOP-18    | Non-small cell lung carcinoma | Lung    | Carcinoma  |
|                    | 0.431 | 0.045  | NCI-H187  | Small cell lung carcinoma | Lung    | Carcinoma  |
| Quercetin          | 0.429 | 0.004  | CWR22R    | Prostate carcinoma epithelial cell line | Prostate | Carcinoma  |
|                    | 0.458 | 0.079  | Hs 683    | Oligodendrogloma    | Brain  | Glioma     |
|                    | 0.401 | 0.029  | HOP-18    | Non-small cell lung carcinoma | Lung    | Carcinoma  |
|                    | 0.405 | 0.067  | NCI-H187  | Small cell lung carcinoma | Lung    | Carcinoma  |
| Scaberin           | 0.483 | 0.018  | NCI-H187  | Small cell lung carcinoma | Lung    | Carcinoma  |
|                    | 0.400 | 0.084  | MCF7      | Breast carcinoma    | Breast  | Carcinoma  |

Conclusion
Based on docking analysis results, the complex of Bcl-2 anti-apoptotic protein with five bioactive compounds of *Cyperus rotundus* such as apigenin, aureusidin, luteolin, quercetin, and scaberin showed a binding affinity score close to obatoclax score. Where the binding affinity of apigenin, luteolin, and quercetin against Bcl-xl was better than obatoclax complex with bcl-xl. The binding interaction that formed between bioactive compounds and both anti-apoptotic proteins demonstrated more number of hydrogen and hydrophobic interactions. These results showed that investigated bioactive compounds were the potential to become an anti-apoptotic protein inhibitor. It is also supported by the prediction of cytotoxicity analysis results of investigated bioactive compounds on various tumor cell lines.

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