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*Original Article*

# Anti-breast cancer potential activity of *Phaleria macrocarpa* (Scheff.) Boerl. leaf extract through *in silico* studies

[Potencial actividad contra el cáncer de mama del extracto de hoja de *Phaleria macrocarpa* (Scheff.) Boerl. mediante estudios *in silico*]

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**Abstract**

*Context*: The development of apoptotic agent from natural plant products may have a beneficial effect as a promising candidate for cancer therapy. The study about the efficacy of *Phaleria macrocarpa* leaves on breast cancer is still limited.

*Aims*: To elucidate the molecular mechanisms underlying the anti-breast cancer activity of *P. macrocarpa* leaves extract by *in silico* analysis.

*Methods*: The compounds of the ethanol extract of *P. macrocarpa* were identified by Liquid Chromatography–High Resolution Mass Spectrometry (LC-HRMS) analysis. Fourteen bioactive compounds of *P. macrocarpa* leaf were analyzed to determine the biological activity using Prediction of Activity Spectra for Substances (PASS) server. The network analysis was analyzed using STRING (https://string-db.org/). Twelve selected compounds were docked with several protein targets, including caspase 3, Bax and Bcl-2. Molecular docking was done by Pyrx 0.8 software and visualized by Discovery Studio software. The pharmacological properties of investigated bioactive compounds were analyzed using the SwissADME web server.

*Results*: The twelve from fourteen bioactive compounds of *P. macrocarpa* leaf have anticancer properties and might be expected to involve in p53 and PI3K/Akt signaling pathways related to cancer. The molecular docking result showed that sesamin from the lignans group has the best binding affinity to caspase-3 and Bax. Meanwhile, corymboside from the flavonoid group has the best binding affinity to Bcl-2.

*Conclusions*: The bioactive compounds of *P. macrocarpa* leaves extract might potentially modulate apoptosis and cell growth. Further research should be performed to validate the activity of *P. macrocarpa* bioactive compounds for target cancer development.

*Keywords*: anticancer activity; binding affinity; bioactive compound; molecular docking; *Phaleria macrocarpa*.

**Resumen**

*Contexto*: El desarrollo de un agente apoptótico a partir de productos vegetales naturales puede tener un efecto beneficioso como candidato prometedor para la terapia del cáncer. El estudio sobre la eficacia de las hojas de *Phaleria macrocarpa* en el cáncer de mama aún es limitado.

*Objetivos*: Dilucidar los mecanismos moleculares que subyacen a la actividad anticancerígena del extracto de hojas de *P. macrocarpa* mediante análisis *in silico*.

*Métodos:* Los compuestos del extracto etanólico de *P. macrocarpa* se identificaron mediante análisis de cromatografía líquida-espectrometría de masas de alta resolución (LC-HRMS). Catorce compuestos bioactivos de la hoja de *P. macrocarpa* fueron analizados para determinar la actividad biológica utilizando el servidor de Predicción de Espectros de Actividad para Sustancias (PASS). El análisis de la red se analizó utilizando STRING (https://string-db.org/). Se acoplaron doce compuestos seleccionados con varias dianas proteicas, incluida la caspasa 3, Bax y Bcl-2. El acoplamiento molecular se realizó con el software Pyrx 0.8 y se visualizó con el software Discovery Studio. Las propiedades farmacológicas de los compuestos bioactivos investigados se analizaron utilizando el servidor web SwissADME.

*Resultados*: Doce de los catorce compuestos bioactivos de la hoja de *P. macrocarpa* tienen propiedades anticancerígenas y se puede esperar que participen en las vías de señalización de p53 y PI3K/Akt relacionadas con el cáncer. El resultado del acoplamiento molecular mostró que la sesamina del grupo de los lignanos tiene la mejor afinidad de unión a la caspasa-3 y Bax. Mientras tanto, el corimbósido del grupo flavonoide tiene la mejor afinidad de unión a Bcl-2.

*Conclusiones*: Los compuestos bioactivos del extracto de hojas de *P. macrocarpa* podrían potencialmente modular la apoptosis y el crecimiento celular. Se deben realizar más investigaciones para validar la actividad de los compuestos bioactivos de *P. macrocarpa* para el desarrollo de dianas terapéuticas contra el cáncer.

*Palabras Clave*: acoplamiento molecular; actividad anticancerígena; afinidad de unión; compuesto bioactivo; *Phaleria macrocarpa*.

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## INTRODUCTION

Breast cancer is one of the most malignancies in women, which is rapidly growing around the world (Ghoncheh et al., 2016). Based on the Global Cancer Observatory (2020), the number of new cases in 2020 accounts for 11.7% of all cancer types. Surprisingly, breast cancer is still the most common cancer in women, followed by lung, colorectum, prostate, stomach, liver, cervix, and esophagus cancer. The incidence and mortality of breast cancer are highly found in developed countries. In Indonesia, about 65,858 women, or 16.6 of all cancer types, were diagnosed with breast cancer (Global Cancer Observatory, 2020). Due to the side effects of chemotherapeutic anticancer drugs, the development of new strategies for breast cancer might be considered (Saeidnia and Abdollahi, 2014; Vasan et al., 2019). The use of novel drug discovery from natural sources can control cancer cells. A total of 80% of people in developing countries used traditional medicine to maintain their health (Unnati et al., 2013).

*Phaleria macrocarpa* (Scheff.) Boerl., which belongs to the family *Thymelaeaceae*, is originated from tropical countries such as Indonesia and Malaysia (Altaf et al., 2013). This plant has been traditionally used to treat numerous diseases such as diabetes, cancer, rheumatism, hypertension, and kidney disorder (Ramdani et al., 2017). The leaf extract of *P. macrocarpa* could increase the expression of NKG2D and CD-122, which then enhances the activity of natural killer cells (NKC) of the spleen (Ghufron et al., 2008). The previous study by Hartati (2005) found that the methanol extract of *P. macrocarpa* contains Phalerin, a new benzophenoic glucoside. Phalerin from *P. macrocarpa* exhibited a cytotoxicity effect on the myeloma cell line by $IC_{50}$ of 83 µg/mL (Hartati et al., 2005). The ethyl acetate extract of *P. macrocarpa* also showed a cytotoxicity effect against leukemia L1210 cells (Katrin et al., 2011). Several studies have been reported the anticancer activity of other parts of *P. macrocarpa*, such as seed (Lay et al., 2014) and fruit (Ramdani et al., 2017). However, the study about the efficacy of ethanol extract of *P. macrocarpa* leaves on breast cancer and their mechanism is still limited.

Some protein is known to play the pivotal roles during apoptosis. The intrinsic pathway of apoptosis involved mitochondria, which regulated by key proteins correlated with apoptosis, such as Bax and Bcl-2. Further, it will activate caspase-3 drives to proteolytic activation of downstream effector, resulting in cell death. The discovery of a novel compound as an anticancer is challenging throughout the world (Agustina et al., 2020). The apoptotic-agent development may have a beneficial effect as a promising candidate for cancer therapy based on its critical role in treating cancer. The present study elucidated the molecular mechanisms underlying the anti-breast cancer activity of *P. macrocarpa* leaves extract by *in silico* analysis.

## MATERIAL AND METHODS

### Plant material

The leaf of *P. macrocarpa* was collected from UPT. Balai Materia Medika, Batu, East Java, Indonesia (7°52'01.2"S and 112°31'13.2"E) in September 2020 and air-dried at room temperature. Taxonomic identification was confirmed and deposited by UPT. Balai Materia Medika, Batu, East Java, Indonesia with determination no. 074/384A/102.7/2020. A total of 100 g of dried leaf were grounded to a fine powder and then macerated with 1000 mL of ethanol at room temperature for 24 h. The extracts were concentrated using a rotary evaporator and stored at 4°C till further analysis.

### Liquid Chromatography–High Resolution Mass Spectrometry (LC-HRMS) analysis

Before injected into the LC-HRMS instrument (Thermo Scientific Dionex Ultimate™ 3000 RSLC-nano with microflow meter, Thermo Fisher Scientific Inc., USA), the extract was diluted with its solvent (polar). Dilution was performed by adding the 0.1% ethanol solvent with the final volume of 1500 µL. The sample was mixed by vortex for 2 min and spindown at 6000 rpm for 2 min. The supernatant was filtered using a syringe filter 0.22

µm and then replaced in the vial. The sample was processed into an autosampler (Thermo Fisher Scientific Inc., USA) and injected in LC-HRMS.

The LC-HRMS analysis, the column oven was maintained at 30°C, the injection volume was 100 mL, the analytical column was Hypersil GOLD aQ (Hypersil GOLD™ aQ C18 Polar Endcapped HPLC Columns, Thermo Fisher Scientific Inc., USA) 50 × 1 mm × 1.9 µ particle size and solvent A = 0.1% formic acid in water and solvent B = 0.1% formic acid in acetonitrile were used. The analytical flow rate was 40 mL/min with a run time of 30 min. The positive ion mode detection using a mass spectrometer (Q Exactive™ Orbitrap Mass Spectrometers (MS), Thermo Fisher Scientific Inc., USA) was used. The compounds were detected using Compound Discoverer software with mzCloud MS/MS library (https://www.mzcloud.org/).

**Biological activity analysis by PASS server**

Fourteen bioactive compounds of *P. macrocarpa* leaf (LC-HRMS analysis results) were analyzed the biological activity using PASS (Prediction of Activity Spectra for Substances, http://www.pharmaexpert.ru/passonline/) server. The canonical smiles of investigated bioactive compounds were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and inserted into the PASS server. A list of biological activity will appear along with the Pa (to be active) and Pi (to be inactive) values. In this study, the Pa value was set at cut-off Pa > 0.5. Pa value represents the activity probability of compounds, where the higher the Pa value, the more significant probability of compounds' activity. Compounds with apoptotic-related activity (Pa > 0.5) were selected and used for molecular docking.

**Ligand-protein interaction and network analysis**

The network analysis to displays the certain pathway involved in cancer were analyzed using STRING (https://string-db.org/) after evaluating the protein-protein interaction by BioGRID Database (https://biogrid.org/). To predict the anticancer properties of *P. macrocarpa* leaves extract, which interferes in the apoptosis signaling pathway, we selected the compounds based on their potency from PASS server results. Ligands and protein interaction networks were then evaluated using the STITCH database (http://stitch.embl.de/).

**Molecular docking and visualization**

Molecular docking was done with twelve selected compounds that showed apoptotic-related activity analyzed by PASS (Table 1). Selected bioactive compounds as ligands were retrieved from PubChem and prepared to minimize the energy using open babel of PyRx software. Protein target of this study were caspase 3 (PDB ID: 3DEI), Bax (PDB ID: 1F16), and Bcl-2 (PDB ID: 1GJH), that obtained from RCSB Protein Data Bank (https://www.rcsb.org/). The proteins were prepared to remove water molecule and unwanted ligand using Discovery Studio 16 software. Molecular docking was done using Pyrx 0.8. Ligands docking to caspase 3 was set to x= -40.5019, y = -2.5866, and z = -15.9094 with dimension (Angstrom) x = 27.9942, y = 25.9985, and z = 14.5592. Ligands docking to Bax protein was set to x = -1.9040, y = 15.4728, and z = -14.5936 with dimension (Angstrom) x = 24.7985, y = 15.7095, and z = 13.3525. Ligands docking to Bcl-2 protein was set to x = -10.5173, y = 3.9012, z = -0.7681 with dimension (Angstrom) x = 10.9910, y = 25.3907, and z = 14.0965. The visualization of docking results was done using Discovery Studio software.

**Pharmacological properties analysis**

The pharmacological properties of investigated bioactive compounds were analyzed using the SwissADME web server (http://www.swissadme.ch/). The canonical smiles of compounds that were retrieved from PubChem were inserted into SwissADME. The pharmacological characteristics and properties were listed on the server.

## RESULTS

### Compound identification by LC-HRMS

The present study identifies the bioactive compounds of *P. macrocarpa* leaves extract. The LC-HRMS analysis revealed that there were 14 bioactive compounds in the ethanolic extract of *P. macrocarpa* leaves extract (Fig. 1), including one alkaloid (trigonelline), five flavonoids (apigenin, corymboside, glycitein, sakuranetin, sterubin), five terpenoid (ageratriol, betulin, cafestol, carvone, and nootkatone), two lignans (matairesinol and sesamin), and one coumarin (fraxetin).

### Apoptotic-related activity screening

The biological activity of *P. macrocarpa* leaf was screened using the PASS server. The results showed that the biological activity of compounds related to apoptosis was at least caspase 3 stimulant, TP53 expression enhancer, anti-carcinogenic, antineoplastic, apoptosis agonist, and caspase 8 stimulants (Fig. 2). Compounds with mentioned activity were selected and used for further analysis. There were two values of probability obtained from PASS analysis, Pa (to be active) and Pi (to be inactive). The higher Pa value than Pi, the higher probability of compound biological activity. The study used cut-off Pa > 0.5 to obtain probability > 50% (Lagunin et al., 2018) of compounds apoptosis activity.

There were 12 selected compounds of *P. macrocarpa* leaves extract, which were involved in the cancer regulation pathway. Corymboside showed the highest TP53 expression enhancer and anti-carcinogenic activity (Pa = 0.941 and 0.872 respectively), among others. Betulin showed the highest caspase 3 stimulant activity (Pa = 0.974) among others. Ageratriol showed the highest antineoplastic activity (Pa = 0.945) among others. Apigenin showed the highest apoptosis agonist activity (Pa = 0.847), among others. Our current result discovered that the bioactive of *P. macrocarpa* leaves extract may be a promising candidate to regulate cancer-associated with apoptosis based on the database server.

### Pathway analysis and protein interaction

Apoptosis resistance and proliferative activity were known as cancer hallmarks. Thus, the development of anticancer drugs mainly focused on caspase stimulants or anti-proliferative. Cell signal transduction via PI3K/Akt/mTOR signaling pathway was considered involved in fundamental processes of cancer development (Ortega et al., 2020). In contrast, p53, known as the guardian genome, was 50% inactive in human cancer. Further, the disturbances of p53 could worsen the survival rate of a cancer patient (Stegh, 2012). Here we investigated the protein involved in cancer using the STRING database (Fig. 3A). The p53 signaling shared two genes with PI3K/Akt signaling pathway in cancer (Fig. 3B). Interestingly, caspase-3 and caspase-8 only participated in the p53 signaling pathway (Fig. 3A-B). PI3K/Akt signaling pathway is a cell signaling regulated in growth, proliferation, survival, and cell metabolism (Guerrero-Zotano et al., 2016). Interestingly, the previous study demonstrated overexpressed PI3K/Akt signaling pathways up to 60% in the human tumor (Engelman, 2009).

Further, to reveal the direct effect of *P. macrocarpa* bioactive compounds, we analyzed using the STITCH database. Our present study described that apigenin, carvone, cafestol, and fraxetin might be involved in p53 and PI3K/Akt signaling pathways related to cancer (Fig. 4). Surprisingly, the network analysis shows no interaction between half of *P. macrocarpa* bioactive compounds in p53 and PI3K/Akt signaling pathways related to cancer. We assumed that it might be because the database is still not updated yet or there are no reports yet associated with each compound. Apigenin is the most ligand with many interactions, particularly with TP53, mTOR, AKT1, BAX, BCL2, CASP 8, CASP 3 in the cancer pathways (Fig. 4). Apigenin activate caspase-3 followed by cleavage of caspase-6, -7, and -8 to induced apoptosis via mitochondria/caspase pathway in breast cancer (Choi and Kim, 2009). Apigenin also could be a potent inhibitor in PI3K/Akt signaling pathway due to its action as an inhibitor signal transduction in breast cancer cells (Seo et al., 2015).

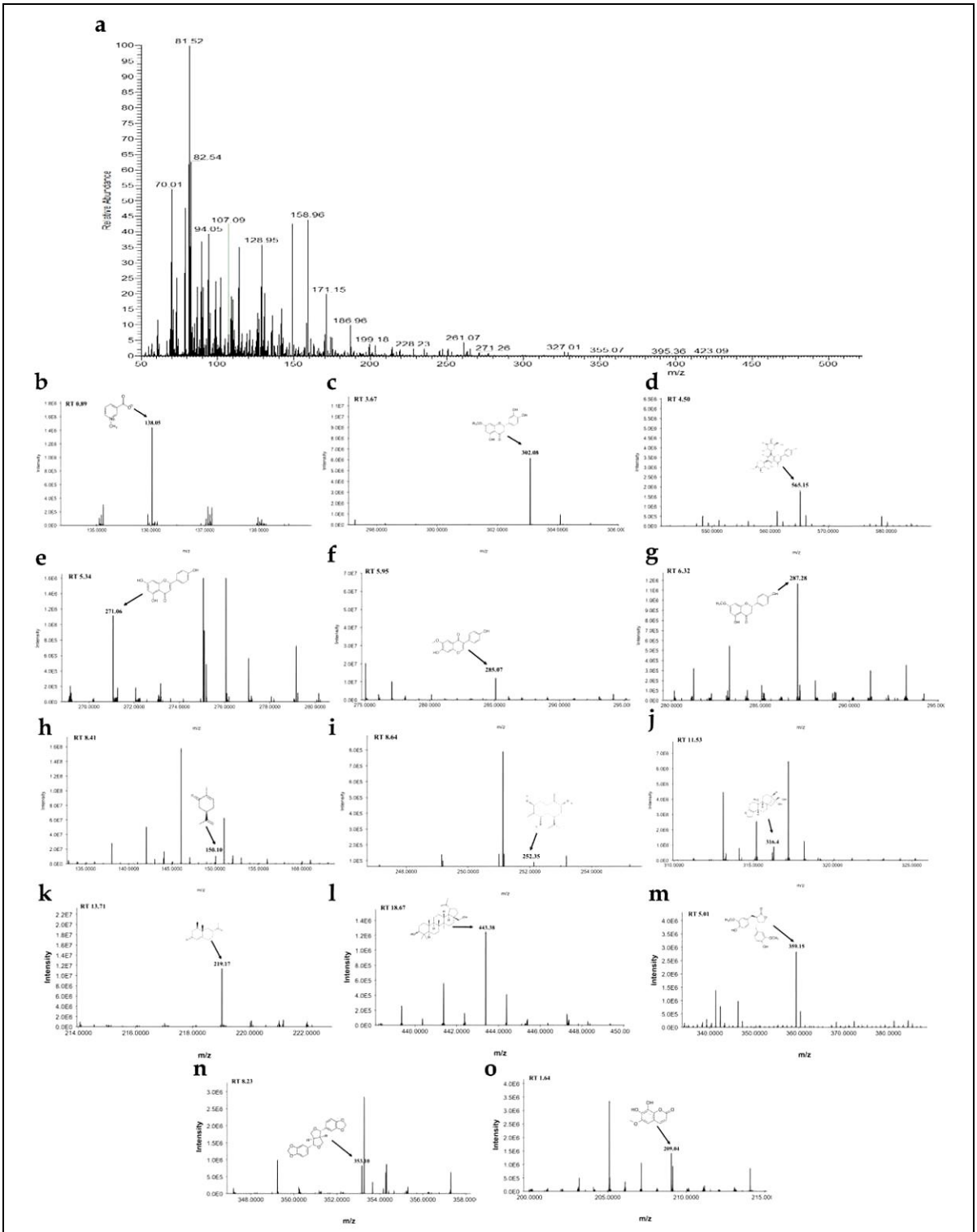



**Figure 1.** LC-HRMS spectrum of bioactive compounds in the ethanolic extract of *P. macrocarpa* leaves extract.

**(a)** The LC-HRMS chromatogram was carried out using (Thermo Scientific Dionex Ultimate™ 3000 RSLCnano (Thermo Fisher Scientific Inc., USA). Fourteen mass spectra were obtained and shown b-o. **(b)** Trigonelline, **(c)** sterubin, **(d)** corymboside, **(e)** apigenin, **(f)** glycitein, **(g)** sakuranetin, **(h)** carvone, **(i)** ageratriol, **(j)** cafestol, **(k)** nootkatone, **(l)** betulin, **(m)** matairesinol, **(n)** sesamin, and **(o)** fraxetin were detected based on its best match by mzCloud MS/MS library.

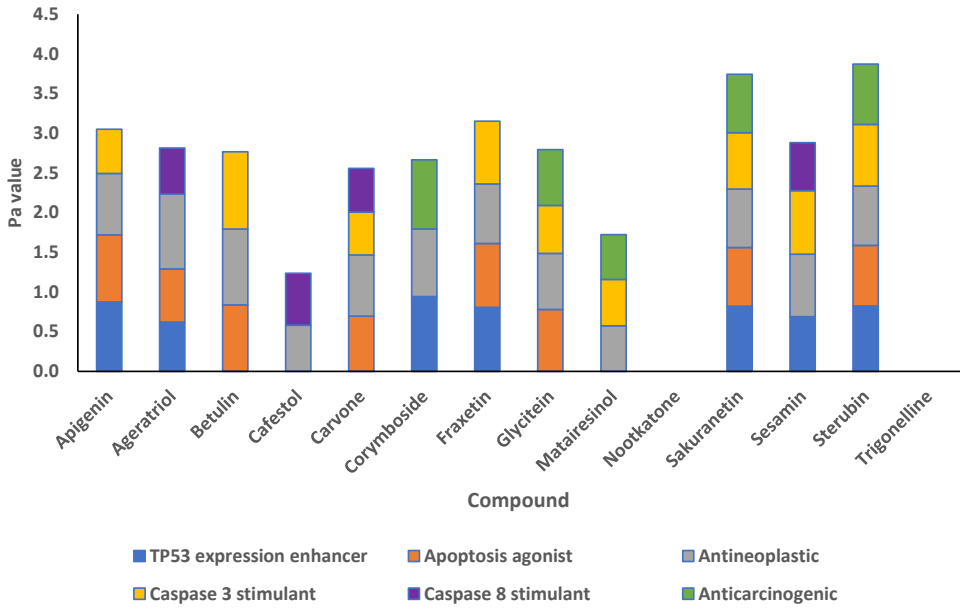
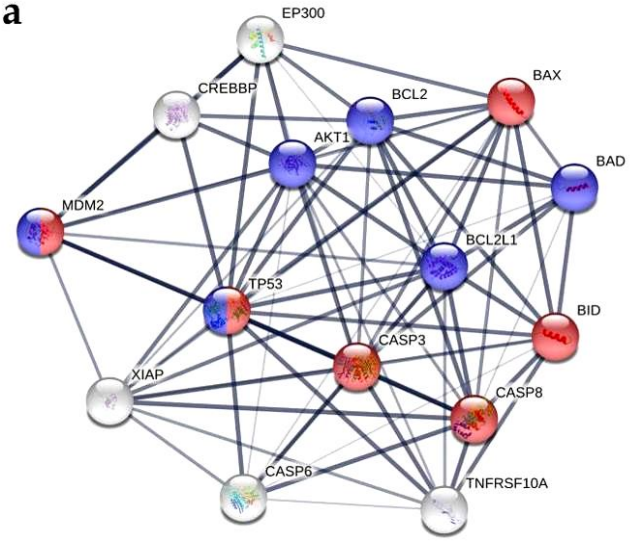



**Figure 2.** Apoptotic activity of selected *P. macrocarpa* leaves bioactive compounds.

**a**




**b**

| Pathway | False Discovery | Genes |
|---|---|---|
| p53 signaling pathway | $1.01 \times 10^{-10}$ | MDM2, TP53, BAX, BID, CASP8, CASP3 |
| PI3K-Akt signaling pathway | $5.4 \times 10^{-7}$ | MDM2, AKT1, BAD, BCL2, BCL2L1, TP53 |

**Figure 3.** Protein-protein interaction network in the apoptosis signaling pathway.

**(a)** The protein-protein interaction network is based on the STRING database, which involved the p53 signaling pathway (red nodes) and PI3K-Akt signaling pathways (blue nodes). **(b)** The genes that participate in the p53 and PI3K-Akt signaling pathways following with the false discovery rates. The boldness for each line represents the number of evidence that has been stored on the database.

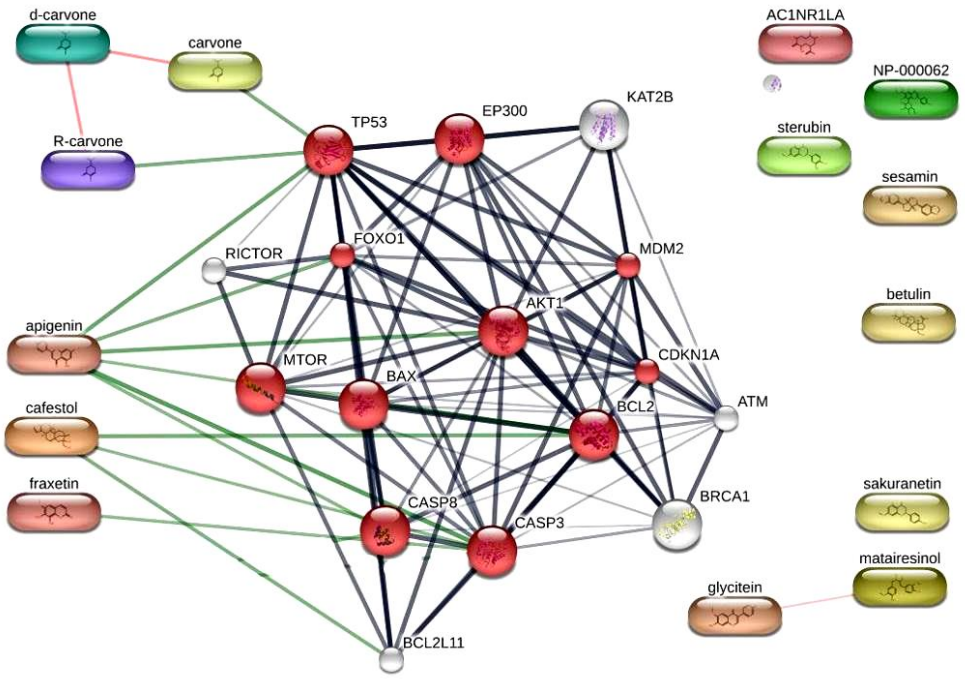



**Figure 4.** Ligand and protein interaction in p53 and PI3K-Akt signaling pathways related to cancer pathways (red nodes).

The bioactive compounds of *P. macrocarpa* leaf as ligands. The green line is considered as the interaction between the ligands to the target protein. The red lines are considered as the relationship between ligands. The black lines are considered as the protein-protein interaction. The boldness for each line represents the number of evidence that have been stored on the database. NP000062 is the synonym of corymboside; AC1NR1LA is the synonym of ageratriol.

## Molecular docking analysis

The molecular docking analysis showed that the highest binding affinity against caspase-3 and Bax was found in the sesamin compound (-9.5 and -7.8 kcal/mol, respectively). While the highest binding affinity against Bcl-2 was found in corymboside (-7.3 kcal/mol) (Table 1).

## Molecular docking of investigated bioactive compounds against Caspase 3 protein

Based on molecular docking analysis (Fig. 5), the binding energy of venetoclax and caspase 3 protein complex was -10.2 kcal/mol that involved several types of interaction via amino acid residues (Table 2). There were 8 hydrogen bond interactions (four conventional hydrogen bonds, three carbon-hydrogen bonds, and a Pi-donor hydrogen bond) and 11 hydrophobic (a Pi-sigma, six alkyl, and five Pi-alkyl). The involvement of amino acid residues in this interaction was Arg147, Tyr197, Gly125, Lys137, Glu124, Tyr195, Pro201, and Leu136. These residues were then posed to be the key amino acid residues in the induction of caspase 3 protein by venetoclax.

Cafestol and caspase 3 complex formed a hydrogen bond (conventional hydrogen bond and seven hydrophobic (a Pi-Pi T-shaped, two alkyl, and four Pi-alkyl) interaction. This complex's hydrogen bond interaction formed via OCS163 residue, where the hydrophobic bond was Phe256, Leu168, Tyr204, Phe256, and Leu168. However, the binding of apigenin against caspase 3 protein formed two hydrogen bonds (conventional hydrogen bond and Pi-donor hydrogen bond), other (Pi-sulfur), and four hydrophobic (two Pi-Pi T-shaped and two Pi-alkyl) that involved several residues, including Leu168, Cys170, and Phe256. Sesamin, betulin, and corymboside interaction with caspase 3 showed the lowest binding affinity than other bioactive compounds and closest to venetoclax binding energy, -9.5, -8.6, and -8.0 kcal/mol, respectively. Sesamin might be a potential candidate to modulate apoptosis. Sesamin activated caspase

3, which has a pivotal role in the last step of apoptosis and cell growth (Siao et al., 2015; Xu et al., 2015). Sesamin and caspase 3 complex formed two hydrogen bonds, an electrostatic, and three hydrophobic interactions. Betulin with higher binding energy interacted with caspase 3 via two hydrogen bonds and four hydrophobic interactions. However, corymboside formed four hydrogen bonds and hydrophobic interaction with caspase 3 protein.

Caspase 3 protein (PDB ID: 3DEI) active site was at key residues of Cys163 and His121 (Tawa et al., 2004). These residues were involved in the catalytic dyad. The catalytic activity of this protein via both amino acids mediated the pre-activation of caspase 3 in cells. Betulin found to interact with a key residue His121 as two hydrophobic interaction that was posed to be involved in caspase 3 (Table 2). His121 also reported being a key residue of caspase active site with other residues including Arg207, Asn208, and Ser209 that can bind with chalcone 9X molecule and formed H-bond simultaneously (Dong et al., 2018). The interaction could change the conformation of caspase 3 protein to be mature or cleaved caspase 3. However, the study found no mentioned residue involved in the complex interaction of investigated bioactive compounds against caspase 3 protein similar to the interaction of venetoclax and caspase 3 complexes.

**Table 1.** The binding affinity of 16 selected compounds of *P. macrocarpa* based on LC-HRMS results.

| Compounds | Binding affinity (kcal/mol) | | |
|---|---|---|---|
| | Caspase-3 | Bcl-2 | Bax |
| **Control drugs** | | | |
| Venetoclax (CID 49846579) | -10.2 | -9.4 | -8.8 |
| **Flavonoid** | | | |
| Apigenin | -7.2 | -6.3 | -6.6 |
| Corymboside | -8.0 | -7.3 | -6.3 |
| Glycitein | -7.5 | -5.5 | -6.8 |
| Sakuranetin | -7.3 | -6.4 | -6.7 |
| Sterubin | -7.5 | -6.7 | -6.8 |
| **Terpenoid** | | | |
| Ageratriol | -6.1 | -5.4 | -6.3 |
| Betulin | -8.6 | -6.4 | -6.9 |
| Cafestol | -7.7 | -6.1 | -7.2 |
| Carvone | -5.8 | -4.4 | -5.3 |
| **Lignans** | | | |
| Matairesinol | -8.2 | -6.4 | -6.5 |
| Sesamin | -9.5 | -6.9 | -7.8 |
| **Coumarin** | | | |
| Fraxetin | -6.1 | -5.2 | -5.7 |

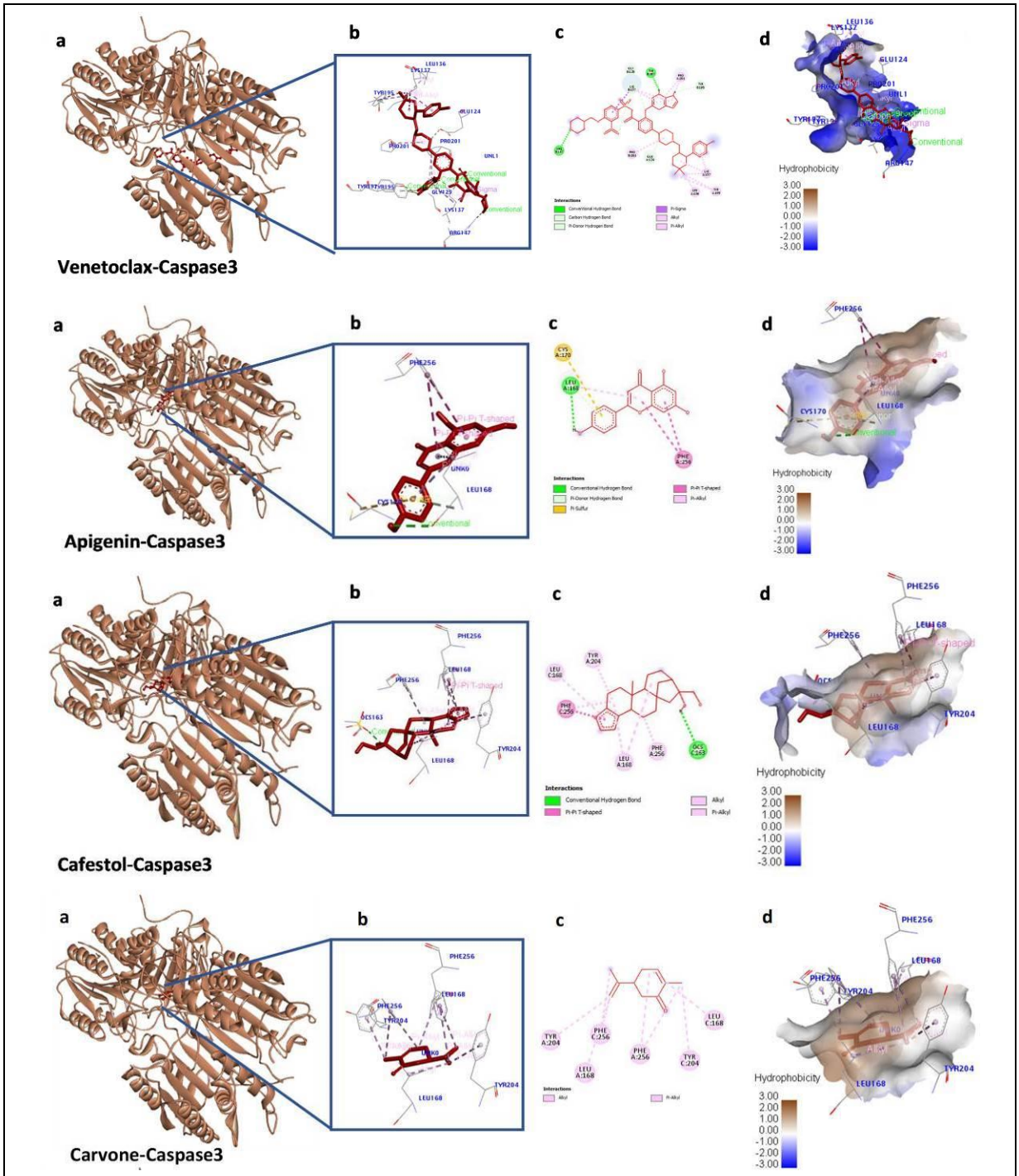



**Figure 5.** Bioactive compounds interaction against caspase 3 protein.

**(a)** Overview ligand-protein complex, **(b)** the active site of ligand-protein complex, **(c)** 2D structure of the ligand-protein complex, **(d)** hydrophobicity of ligand-protein complex. Brown solid ribbon was caspase 3, and red ball-and-stick was a bioactive compound.

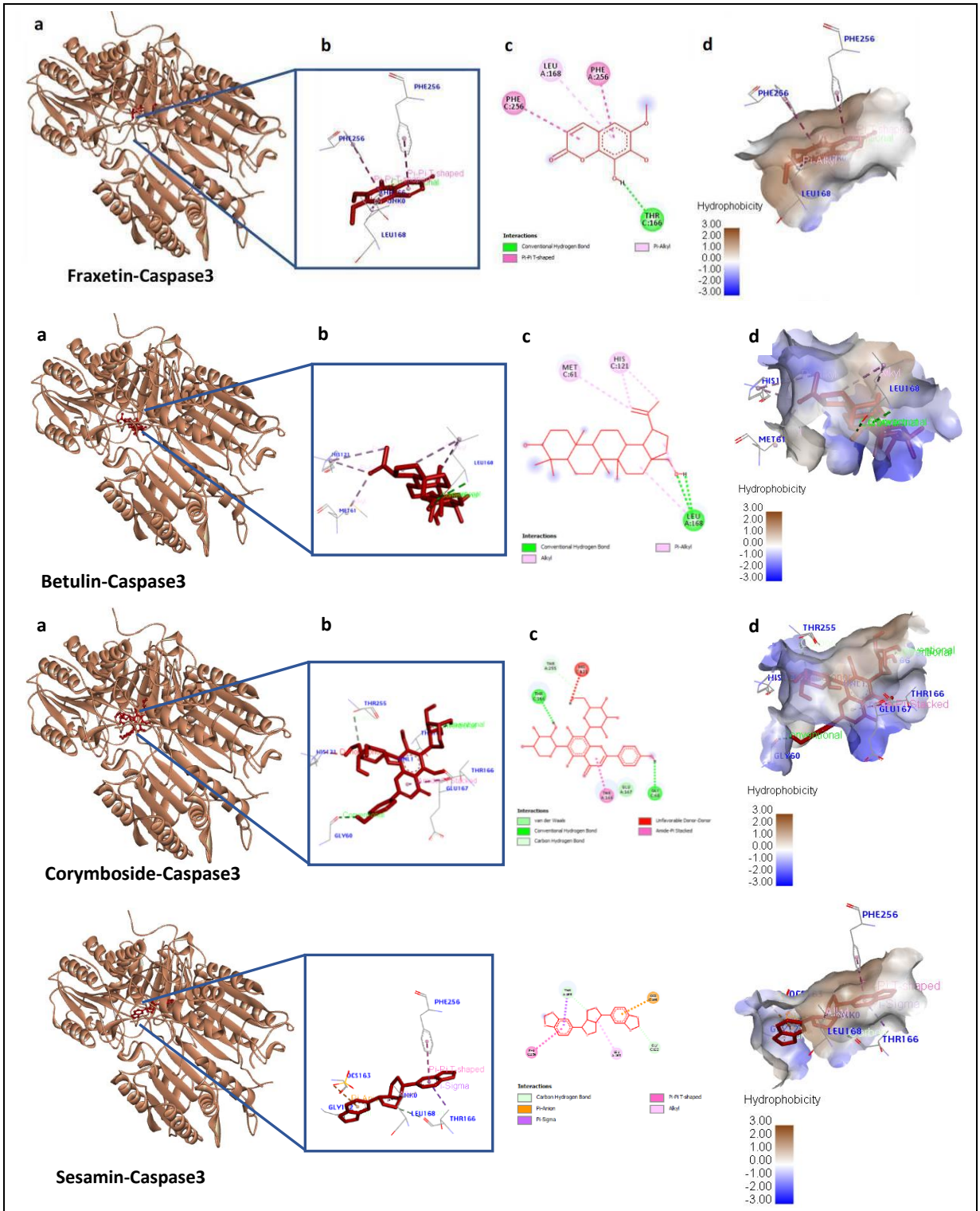



**Figure 5.** Bioactive compounds interaction against caspase 3 protein (continued…).

**(a)** Overview ligand-protein complex, **(b)** the active site of ligand-protein complex, **(c)** 2D structure of the ligand-protein complex, **(d)** hydrophobicity of ligand-protein complex. Brown solid ribbon was caspase 3, and red ball-and-stick was a bioactive compound.

**Table 2.** Interaction of venetoclax and investigated bioactive compounds against caspase 3 protein.

| Complexes (binding energy in Kcal/mol) | Interaction | Category | Type |
|---|---|---|---|
| **Venetoclax-caspase 3 (-10.2)** | B:ARG147:NH2 - N:UNL1:O | Hydrogen bond | Conventional hydrogen bond |
| | N:UNL1:H - N:UNL1:O | Hydrogen bond | Conventional hydrogen bond |
| | N:UNL1:H - N:UNL1:O | Hydrogen bond | Conventional hydrogen bond |
| | N:UNL1:HN - B:TYR197:OH | Hydrogen bond | Conventional hydrogen bond |
| | B:GLY125:CA - N:UNL1:O | Hydrogen bond | Carbon hydrogen bond |
| | B:LYS137:CE - N:UNL1:O | Hydrogen bond | Carbon hydrogen bond |
| | N:UNL1:C - A:GLU124:OE2 | Hydrogen bond | Carbon hydrogen bond |
| | N:UNL1:H - B:TYR195 | Hydrogen bond | Pi-donor hydrogen bond |
| | B:LYS137:CG - N:UNL1 | Hydrophobic | Pi-sigma |
| | A:LYS137 - N:UNL1 | Hydrophobic | Alkyl |
| | A:PRO201 - N:UNL1 | Hydrophobic | Alkyl |
| | B:LYS137 - N:UNL1 | Hydrophobic | Alkyl |
| | B:PRO201 - N:UNL1 | Hydrophobic | Alkyl |
| | N:UNL1:C - A:LEU136 | Hydrophobic | Alkyl |
| | N:UNL1:C - A:LYS137 | Hydrophobic | Alkyl |
| | A:TYR195 - N:UNL1:C | Hydrophobic | Pi-alkyl |
| | A:TYR195 - N:UNL1:C | Hydrophobic | Pi-alkyl |
| | N:UNL1 - A:PRO201 | Hydrophobic | Pi-alkyl |
| | N:UNL1 - B:LYS137 | Hydrophobic | Pi-alkyl |
| | N:UNL1 - A:LYS137 | Hydrophobic | Pi-alkyl |
| **Apigenin–caspase 3 (-7.2)** | N:UNK0:H - A:LEU168:O | Hydrogen bond | Conventional hydrogen bond |
| | A:LEU168:N - N:UNK0 | Hydrogen bond | Pi-donor hydrogen bond |
| | A:CYS170:SG - N:UNK0 | Other | Pi-sulfur |
| | A:PHE256 - N:UNK0 | Hydrophobic | Pi-Pi T-shaped |
| | A:PHE256 - N:UNK0 | Hydrophobic | Pi-Pi T-shaped |
| | N:UNK0 - A:LEU168 | Hydrophobic | Pi-alkyl |
| | N:UNK0 - A:LEU168 | Hydrophobic | Pi-alkyl |
| **Cafestol–caspase 3 (-7.7)** | N:UNK0:H - C:OCS163:OD1 | Hydrogen bond | Conventional hydrogen bond |
| | N:UNK0 - C:PHE256 | Hydrophobic | Pi-Pi T-shaped |
| | A:LEU168 - N:UNK0 | Hydrophobic | Alkyl |
| | A:LEU168 - N:UNK0 | Hydrophobic | Alkyl |
| | A:TYR204 - N:UNK0 | Hydrophobic | Pi-alkyl |
| | A:PHE256 - N:UNK0 | Hydrophobic | Pi-alkyl |
| | C:PHE256 - N:UNK0 | Hydrophobic | Pi-alkyl |
| | N:UNK0 - C:LEU168 | Hydrophobic | Pi-alkyl |

**Table 2.** Interaction of venetoclax and investigated bioactive compounds against caspase 3 protein (continued…).

| Complexes (binding energy in Kcal/mol) | Interaction | Category | Type |
|---|---|---|---|
| **Carvone–caspase 3 (-5.8)** | N:UNK0 - C:LEU168 | Hydrophobic | Alkyl |
| | N:UNK0:C - A:LEU168 | Hydrophobic | Alkyl |
| | A:TYR204 - N:UNK0:C | Hydrophobic | Pi-alkyl |
| | A:PHE256 - N:UNK0 | Hydrophobic | Pi-alkyl |
| | A:PHE256 - N:UNK0:C | Hydrophobic | Pi-alkyl |
| | C:TYR204 - N:UNK0:C | Hydrophobic | Pi-alkyl |
| | C:PHE256 - N:UNK0:C | Hydrophobic | Pi-alkyl |
| | C:PHE256 - N:UNK0:C | Hydrophobic | Pi-alkyl |
| **Fraxetin–caspase 3 (-6.1)** | N:UNK0:H - C:THR166:O | Hydrogen bond | Conventional hydrogen bond |
| | A:PHE256 - N:UNK0 | Hydrophobic | Pi-Pi T-shaped |
| | C:PHE256 - N:UNK0 | Hydrophobic | Pi-Pi T-shaped |
| | N:UNK0 - A:LEU168 | Hydrophobic | Pi-alkyl |
| **Betulin–caspase 3 (-8.6)** | A:LEU168:N - N:UNK0:O | Hydrogen bond | Conventional hydrogen bond |
| | N:UNK0:H - A:LEU168:O | Hydrogen bond | Conventional hydrogen bond |
| | N:UNK0 - A:LEU168 | Hydrophobic | Alkyl |
| | N:UNK0 - A:LEU168 | Hydrophobic | Alkyl |
| | N:UNK0:C - C:MET61 | Hydrophobic | Alkyl |
| | C:HIS121 - N:UNK0:C | Hydrophobic | Pi-alkyl |
| | C:HIS121 - N:UNK0:C | Hydrophobic | Pi-alkyl |
| **Corymboside–caspase 3 (-8.0)** | N:UNL1:H - N:UNL1:O | Hydrogen bond | Conventional hydrogen bond |
| | N:UNL1:H - C:THR166:O | Hydrogen bond | Conventional hydrogen bond |
| | N:UNL1:H - C:GLY60:O | Hydrogen bond | Conventional hydrogen bond |
| | N:UNL1:C - A:THR255:OG1 | Hydrogen bond | Carbon hydrogen bond |
| | A:THR166:C,O;GLU167:N - N:UNL1 | Hydrophobic | Amide-Pi stacked |
| **Sesamin–caspase 3 (-9.5)** | N:UNK0:C - A:THR166:O | Hydrogen bond | Carbon hydrogen bond |
| | N:UNK0:C - C:GLY122:O | Hydrogen bond | Carbon hydrogen bond |
| | C:OCS163:OD3 - N:UNK0 | Electrostatic | Pi-anion |
| | A:THR166:CG2 - N:UNK0 | Hydrophobic | Pi-sigma |
| | C:PHE256 - N:UNK0 | Hydrophobic | Pi-Pi T-shaped |
| | N:UNK0 - A:LEU168 | Hydrophobic | Alkyl |

## Molecular docking of investigated bioactive compounds against Bcl-2 protein

The molecular docking results of investigated bioactive compounds and venetoclax against Bcl-2 protein was shown in Fig. 6. Venetoclax was a well-known Bcl-2 inhibitor used to induce apoptosis in the cancer cell. The interaction of venetoclax and Bcl-2 protein formed 4 hydrogen bonds via Ser49, Glu45, and Asp35. This complex also formed halogen interaction via Met16 residue, two electrostatic interactions via Glu13 and Glu50, and

9 hydrophobic interactions via His20, Val36, Met16, Ala32, Pro44, and Trp30. These residues were posed to be the key amino acid residues in the inhibition of Bcl-2 by venetoclax. The binding energy of this complex was -9.4 kcal/mol.

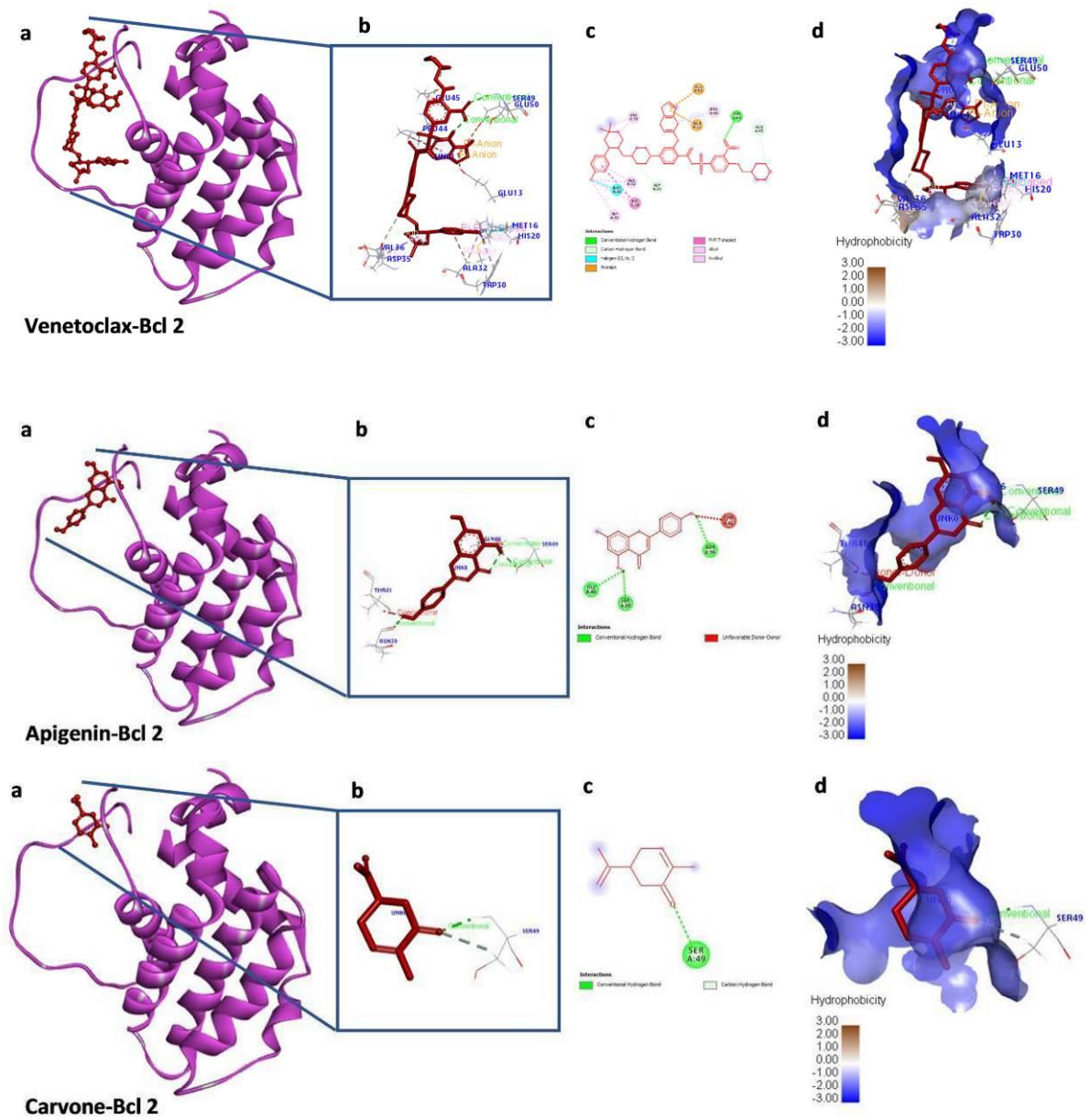



**Figure 6.** Bioactive compounds interaction against Bcl-2 protein.

**(a)** Overview ligand-protein complex, **(b)** the active site of ligand-protein complex, **(c)** 2D structure of the ligand-protein complex, **(d)** hydrophobicity of ligand-protein complex. The solid pink ribbon was Bcl-2, and the red ball-and-stick was a bioactive compound.

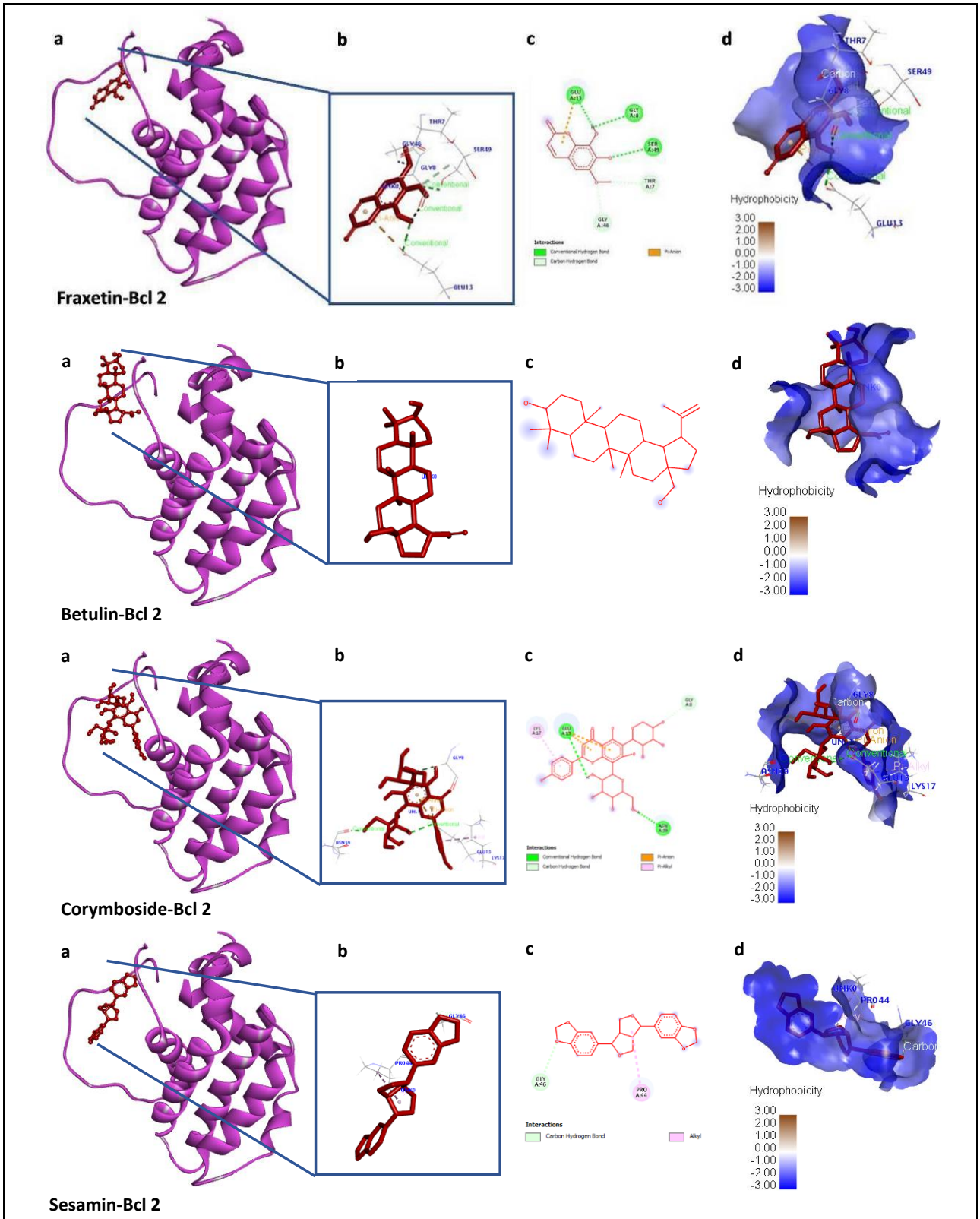



**Figure 6.** Bioactive compounds interaction against Bcl-2 protein (continued…).

**(a)** Overview ligand-protein complex, **(b)** the active site of ligand-protein complex, **(c)** 2D structure of the ligand-protein complex, **(d)** hydrophobicity of ligand-protein complex. The solid pink ribbon was Bcl-2, and the red ball-and-stick was a bioactive compound.

A key residue of Ser49 was involved in several interactions, including Bcl-2 and apigenin as hydrogen bonds, Bcl-2 and carvone as two hydrogen bonds, and Bcl-2 and fraxetin as two hydrogen bonds. A residue Glu13, as venetoclax-Bcl-2 complex key residue also found in the complex of cafestol-Bcl-2 (hydrogen bond), fraxetin-Bcl-2 (hydrogen bond), and betulin-Bcl-2 (a hydrogen bond and two electrostatics). The last key residue, Pro44, was found in the complex of sesamin-Bcl-2 protein as hydrophobic interaction.

The involvement of several key residues in the complex of bioactive compounds against Bcl-2 protein was posed to disturb the activity and inhibit Bcl-2 protein as venetoclax. The study showed that corymboside (-7.3 kcal/mol), and sesamin (-6.9 kcal/mol) still have a weaker binding than venetoclax (-9.4 kcal/mol) (Table 3).

**Molecular docking of investigated bioactive compounds against Bax protein**

Gavathiotis et al. (2008) analyzed the binding site of Bax protein that directly mediated mitochondrial apoptosis initiation. Gavathiotis found several amino acid residues responsible for the induction of Bax activation, such as Lys21, Gln28, Gln32, Arg134, and Glu131. However, the study found that venetoclax interaction with Bax protein involved a key residue such as Gln32 through hydrogen bond interaction (Table 4). Other key amino acids of Bax (PDB ID: 1F16) active site also determined by Liu et al. (2016), Bax activation also mediated by Pro168 and Pro13 amino acid residues. Pro13 was also a residue involved in venetoclax and Bax complex.

Venetoclax was reported to induce apoptosis via Bax activation (Alhoshani et al., 2020). This study posed the mechanism of venetoclax interaction against Bax protein. Venetoclax bind to Bax via 4 hydrogen bonds, an electrostatic, and 11 hydrophobic interactions. The binding energy was -8.8 kcal/mol with residues Pro49, Gln32, Glu44, Ala35, Val50, Leu125, Pro43, Pro130, Pro51, and Ala42 (Table 4). The study found venetoclax involved Gln32 residue, one of the residues crucial in the induction of Bax activation in line with Gavathiotis et al. (2008).

A key residue, Gln32, also involved the interaction of sesamin and Bax protein through a hydrogen bond. The involvement of a key residue Glu44 demonstrated by the complex Bax apigenin and fraxetin as electrostatic interaction, in betulin as two hydrogen bonds, corymboside, and sesamin as hydrogen bond. Apigenin, fraxetin, betulin, and sesamin also showed to involve a key residue Ala35 as hydrophobic interactions. Val50 was found to involve in the complex of carvone and Bax as hydrophobic interaction. Leu125 was only found to involve in apigenin, corymboside, and sesamin interaction against Bax as hydrophobic interaction. Whereas Ala42 was a key residue of Bax that was mostly found in the interaction of investigated bioactive compounds, including apigenin via two hydrophobics, cafestol as four hydrophobics, betulin via three hydrophobics, where carvone, fraxetin, and sesamin as a hydrophobic interaction.

This study showed that sesamin has the lowest binding energy (-7.8 kcal/mol) followed by betulin (-6.9 kcal/mol), and cafestol (-7.2 kcal/mol). The previous study demonstrated that sesamin elevated Bax expression in MCF-7 cells (Siao et al., 2015). Further, sesamin also induces sub-G1 arrest in the cell cycle associated with elevating p53 protein. P53 protein was known to be responsible for genomic instability, and it is crucial to driving cell cycle arrest or apoptosis (Lee et al., 2014; Siao et al., 2015).

Besides, this study showed that apigenin was mostly showed the involvement of key residues in the interaction against Bax protein. Apigenin also demonstrated the lowest binding energy (-6.6 kcal/mol) after cafestol (-7.2 kcal/mol). We assumed that apigenin could induce Bax protein via the involvement of Glu44, Ala35, Leu125, and Ala42 residues. The visualization of molecular docking of its complex was shown in Fig. 7. Our novel finds that the *P. macrocarpa* leaf contains many bioactive compounds beneficial for cancer therapy by induced apoptosis via interaction with caspase-3, Bcl-2, and Bax protein.

**Table 3.** Interaction of venetoclax and investigated bioactive compounds against Bcl-2 protein.

| Complexes (binding energy in Kcal/mol) | Interaction | Category | Type |
|---|---|---|---|
| **Venetoclax–Bcl 2 (-9.4)** | N:UNL1:HN - N:UNL1:O | Hydrogen bond | Conventional hydrogen bond |
| | A:SER49:HN - N:UNL1:O | Hydrogen bond | Conventional hydrogen bond |
| | N:UNL1:C - A:GLU45:O | Hydrogen Bond | Carbon hydrogen bond |
| | N:UNL1:C - A:ASP35:O | Hydrogen bond | Carbon hydrogen bond |
| | A:MET16:O - N:UNL1:Cl | Halogen | Halogen (Cl, Br, I) |
| | A:GLU13:OE2 - N:UNL1 | Electrostatic | Pi-anion |
| | A:GLU50:OE2 - N:UNL1 | Electrostatic | Pi-anion |
| | A:HIS20 - N:UNL1 | Hydrophobic | Pi-Pi T-shaped |
| | N:UNL1:C - A:VAL36 | Hydrophobic | Alkyl |
| | N:UNL1:Cl - A:MET16 | Hydrophobic | Alkyl |
| | N:UNL1:Cl - A:ALA32 | Hydrophobic | Alkyl |
| | A:VAL36 - N:UNL1 | Hydrophobic | Alkyl |
| | A:PRO44 - N:UNL1 | Hydrophobic | Alkyl |
| | N:UNL1 - A:ALA32 | Hydrophobic | Pi-alkyl |
| | A:HIS20 - N:UNL1:Cl | Hydrophobic | Pi-alkyl |
| | A:TRP30 - N:UNL1:Cl | Hydrophobic | Pi-alkyl |
| **Apigenin–Bcl 2 (-6.3)** | N:UNK0:H - A:ASN39:O | Hydrogen bond | Conventional hydrogen bond |
| | N:UNK0:H - A:GLY46:O | Hydrogen bond | Conventional hydrogen bond |
| | N:UNK0:H - A:SER49:OG | Hydrogen bond | Conventional hydrogen bond |
| | N:UNK0:H - N:UNK0:O | Hydrogen bond | Conventional hydrogen bond |
| **Cafestol–Bcl 2 (-6.1)** | N:UNK0:H - A:ASN39:OD1 | Hydrogen bond | Conventional hydrogen bond |
| | N:UNK0:H - A:GLU13:OE2 | Hydrogen bond | Conventional hydrogen bond |
| | N:UNK0:C - A:GLU48:OE1 | Hydrogen bond | Carbon hydrogen bond |
| **Carvone–Bcl 2 (-4.4)** | A:SER49:HN - N:UNK0:O | Hydrogen bond | Conventional hydrogen bond |
| | A:SER49:HB2 - N:UNK0:O | Hydrogen bond | Carbon hydrogen bond |
| **Fraxetin–Bcl 2 (-5.2)** | A:SER49:HG - N:UNK0:O | Hydrogen bond | Conventional hydrogen bond |
| | N:UNK0:H - A:GLY8:O | Hydrogen bond | Conventional hydrogen bond |
| | N:UNK0:H - A:GLU13:OE1 | Hydrogen bond | Conventional hydrogen bond |
| | A:GLY46:HA2 - N:UNK0:O | Hydrogen bond | Carbon hydrogen bond |
| | A:SER49:HB2 - N:UNK0:O | Hydrogen bond | Carbon hydrogen bond |
| | N:UNK0:C - A:THR7:O | Hydrogen bond | Carbon hydrogen bond |
| | A:GLU13:OE2 - N:UNK0 | Electrostatic | Pi-anion |
| **Betulin–Bcl 2 (-6.4)** | - | - | - |

**Table 3.** Interaction of venetoclax and investigated bioactive compounds against Bcl-2 protein (continued…).

| Complexes (binding energy in Kcal/mol) | Interaction | Category | Type |
|---|---|---|---|
| **Corymboside–Bcl 2 (-7.3)** | N:UNL1:H - A:ASN39:O | Hydrogen bond | Conventional hydrogen bond |
| | N:UNL1:H - A:GLU13:OE2 | Hydrogen bond | Conventional hydrogen bond |
| | A:GLY8:HA2 - N:UNL1:O | Hydrogen bond | Carbon hydrogen bond |
| | A:GLU13:OE2 - N:UNL1 | Electrostatic | Pi-anion |
| | A:GLU13:OE2 - N:UNL1 | Electrostatic | Pi-anion |
| | N:UNL1 - A:LYS17 | Hydrophobic | Pi-alkyl |
| **Sesamin–Bcl 2 (-6.9)** | A:GLY46:HA1 - N:UNK0:O | Hydrogen bond | Carbon hydrogen bond |
| | A:PRO44 - N:UNK0 | Hydrophobic | Alkyl |

**Table 4.** Interaction of venetoclax and investigated bioactive compounds against Bax protein.

| Complexes (binding energy in Kcal/mol) | Interaction | Category | Type |
|---|---|---|---|
| **Venetoclax–Bcl 2 (-9.4)** | N:UNL1:HN - N:UNL1:O | Hydrogen bond | Conventional hydrogen bond |
| | A:SER49:HN - N:UNL1:O | Hydrogen bond | Conventional hydrogen bond |
| | N:UNL1:C - A:GLU45:O | Hydrogen bond | Carbon hydrogen bond |
| | N:UNL1:C - A:ASP35:O | Hydrogen bond | Carbon hydrogen bond |
| | A:MET16:O - N:UNL1:Cl | Halogen | Halogen (Cl, Br, I) |
| | A:GLU13:OE2 - N:UNL1 | Electrostatic | Pi-anion |
| | A:GLU50:OE2 - N:UNL1 | Electrostatic | Pi-anion |
| | A:HIS20 - N:UNL1 | Hydrophobic | Pi-Pi T-shaped |
| | N:UNL1:C - A:VAL36 | Hydrophobic | Alkyl |
| | N:UNL1:Cl - A:MET16 | Hydrophobic | Alkyl |
| | N:UNL1:Cl - A:ALA32 | Hydrophobic | Alkyl |
| | A:VAL36 - N:UNL1 | Hydrophobic | Alkyl |
| | A:PRO44 - N:UNL1 | Hydrophobic | Alkyl |
| | N:UNL1 - A:ALA32 | Hydrophobic | Pi-alkyl |
| | A:HIS20 - N:UNL1:Cl | Hydrophobic | Pi-alkyl |
| | A:TRP30 - N:UNL1:Cl | Hydrophobic | Pi-alkyl |
| **Apigenin–Bcl 2 (-6.3)** | N:UNK0:H - A:ASN39:O | Hydrogen bond | Conventional hydrogen bond |
| | N:UNK0:H - A:GLY46:O | Hydrogen bond | Conventional hydrogen bond |
| | N:UNK0:H - A:SER49:OG | Hydrogen bond | Conventional hydrogen bond |
| | N:UNK0:H - N:UNK0:O | Hydrogen bond | Conventional hydrogen bond |

**Table 4.** Interaction of venetoclax and investigated bioactive compounds against Bax protein (continued…).

| Category | Type | | |
|---|---|---|---|
| **Cafestol–Bcl 2 (-6.1)** | N:UNK0:H - A:ASN39:OD1 | Hydrogen bond | Conventional hydrogen bond |
| | N:UNK0:H - A:GLU13:OE2 | Hydrogen bond | Conventional hydrogen bond |
| | N:UNK0:C - A:GLU48:OE1 | Hydrogen bond | Carbon hydrogen bond |
| **Carvone–Bcl 2 (-4.4)** | A:SER49:HN - N:UNK0:O | Hydrogen bond | Conventional hydrogen bond |
| | A:SER49:HB2 - N:UNK0:O | Hydrogen bond | Carbon hydrogen bond |
| **Fraxetin–Bcl 2 (-5.2)** | A:SER49:HG - N:UNK0:O | Hydrogen bond | Conventional hydrogen bond |
| | N:UNK0:H - A:GLY8:O | Hydrogen bond | Conventional hydrogen bond |
| | N:UNK0:H - A:GLU13:OE1 | Hydrogen bond | Conventional hydrogen bond |
| | A:GLY46:HA2 - N:UNK0:O | Hydrogen bond | Carbon hydrogen bond |
| | A:SER49:HB2 - N:UNK0:O | Hydrogen bond | Carbon hydrogen bond |
| | N:UNK0:C - A:THR7:O | Hydrogen bond | Carbon hydrogen bond |
| | A:GLU13:OE2 - N:UNK0 | Electrostatic | Pi-anion |
| **Betulin–Bcl 2 (-6.4)** | - | - | - |
| **Corymboside–Bcl 2 (-7.3)** | N:UNL1:H - A:ASN39:O | Hydrogen bond | Conventional hydrogen bond |
| | N:UNL1:H - A:GLU13:OE2 | Hydrogen bond | Conventional hydrogen bond |
| | A:GLY8:HA2 - N:UNL1:O | Hydrogen bond | Carbon hydrogen bond |
| | A:GLU13:OE2 - N:UNL1 | Electrostatic | Pi-anion |
| | A:GLU13:OE2 - N:UNL1 | Electrostatic | Pi-anion |
| | N:UNL1 - A:LYS17 | Hydrophobic | Pi-alkyl |
| **Sesamin–Bcl 2 (-6.9)** | A:GLY46:HA1 - N:UNK0:O | Hydrogen bond | Carbon hydrogen bond |
| | A:PRO44 - N:UNK0 | Hydrophobic | Alkyl |

### SwissADME analysis

The optimum criteria for bioactive compounds to be consumed orally as if they have the following range of physical parameters, such as molecular weight (150-500 g/mol), polarity (TPSA between 20-130Å2), solubility (log S not more than six), flexibility (not more than nine rotatable bonds), and lipophilicity (XLOGP3 between -0.7 and +5.0) (Daina et al., 2017). Based on these criteria, apigenin, cafestol, fraxetin, and sesamin were met the optimum criteria range. Whereas carvone has very low TPSA < 20, betulin has < XLOGP, and corymboside has higher MW < 500 g/mol, XLOGP >-0.7, and TPSA > 130 Å2 did not meet the optimum criteria of its physicochemical properties (Table 5).

## CONCLUSIONS

In the present study was demonstrated the molecular mechanisms underlying *P. macrocarpa* leaves extract as anti-breast cancer *in silico* analysis. The present study suggested that bioactive compounds from *P. macrocarpa* leaf extract might potentially modulate apoptosis and cell growth. Sesamin from the lignans group was predicted as a strong caspase-3 and Bax stimulant. Meanwhile, corymboside from the flavonoid group was predicted as a Bcl-2 inhibitor. The *P. macrocarpa* bioactive compounds may be expected to interfere in p53 and PI3K/Akt signaling pathways related to cancer to inhibit cell growth and induce apoptosis. Further research should be performed to validate

the activity of *P. macrocarpa* bioactive compounds for target cancer development.

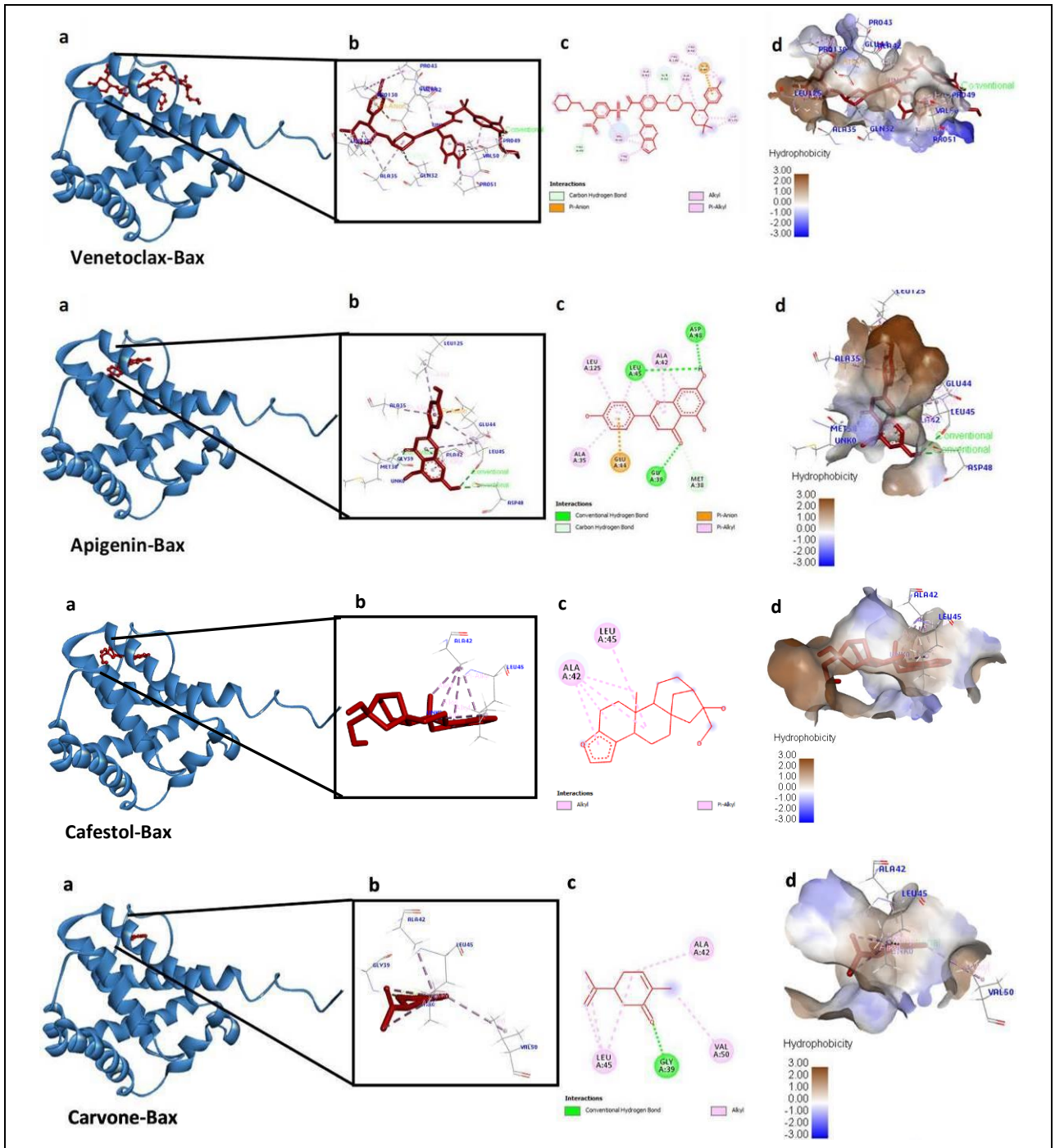



**Figure 7.** Bioactive compounds interaction against Bax protein.

**(a)** Overview ligand-protein complex, **(b)** the active site of ligand-protein complex, **(c)** 2D structure of the ligand-protein complex, **(d)** hydrophobicity of ligand-protein complex. Blue solid ribbon was Bax, and red ball-and-stick was a bioactive compound.

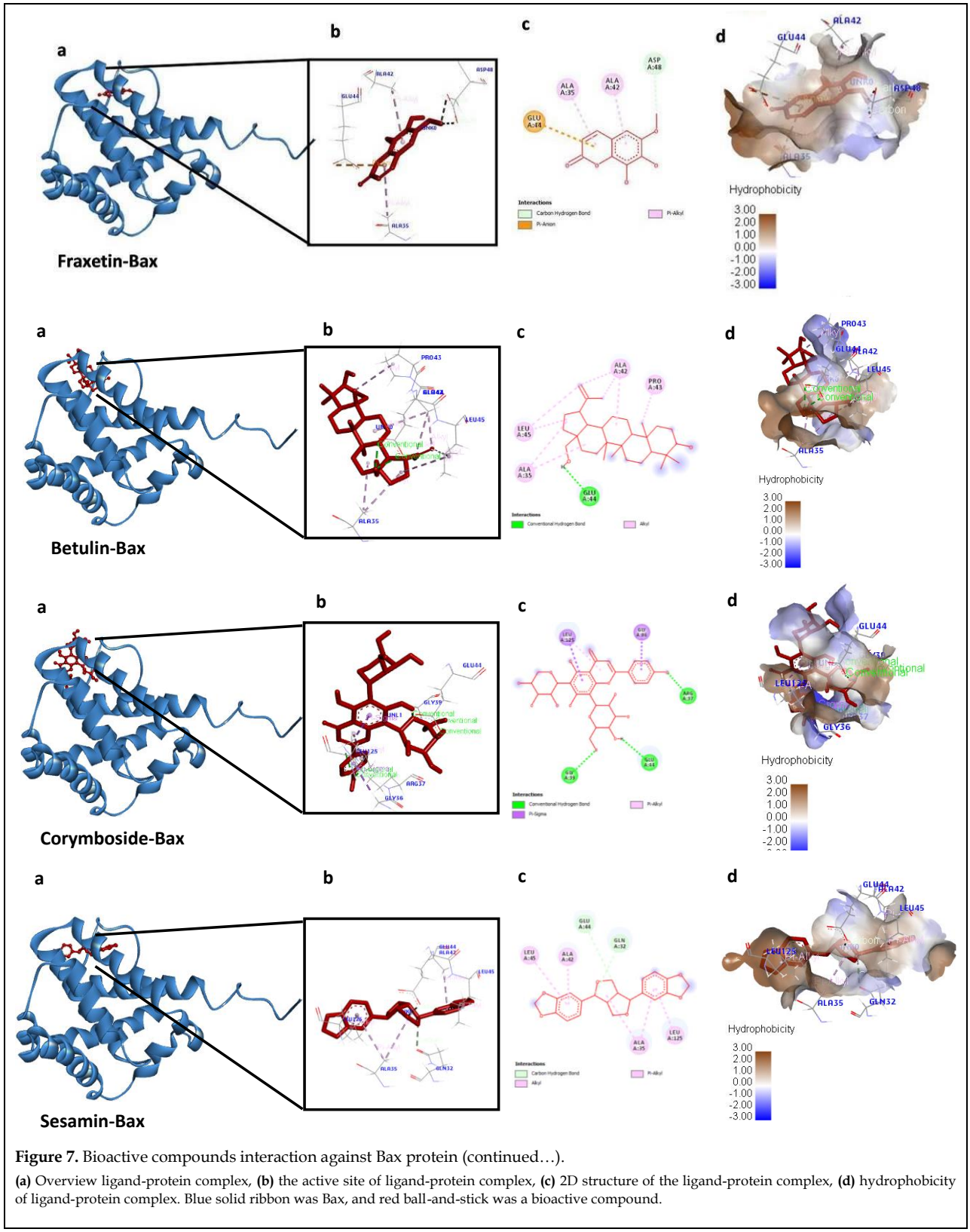



**Figure 7.** Bioactive compounds interaction against Bax protein (continued…).

**(a)** Overview ligand-protein complex, **(b)** the active site of ligand-protein complex, **(c)** 2D structure of the ligand-protein complex, **(d)** hydrophobicity of ligand-protein complex. Blue solid ribbon was Bax, and red ball-and-stick was a bioactive compound.

**Table 5.** Pharmacological properties of investigated bioactive compounds.

| Compound | Mol. Weight (g/mol) | LogP value | H-bond donor | H-bond acceptor | Rotatable bonds | TPSA |
|---|---|---|---|---|---|---|
| **Apigenin** | 270.24 | 3.02 | 3 | 5 | 1 | 90.90 Å² |
| **Cafestol** | 316.43 | 3.28 | 2 | 3 | 1 | 53.60 Å² |
| **Carvone** | 150.22 | 2.71 | 0 | 1 | 1 | 17.07 Å² |
| **Fraxetin** | 208.17 | 1.17 | 2 | 5 | 1 | 79.90 Å² |
| **Betulin** | 442.72 | 8.28 | 2 | 2 | 2 | 40.46 Å² |
| **Corymboside** | 564.49 | -2.19 | 10 | 14 | 4 | 250.97 Å² |
| **Sesamin** | 354.35 | 2.68 | 0 | 6 | 2 | 55.38 Å² |

## CONFLICT OF INTEREST

The authors declare no conflicts of interests.

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| Contribution | Christina YI | Nafisah W | Atho'illah MF | Rifa'i M | Widodo N | Djati MS |
|---|---|---|---|---|---|---|
| Concepts or ideas | x | | | | | |
| Design | x | | | | | |
| Definition of intellectual content | x | | | | | |
| Literature search | | x | | | | |
| Experimental studies | x | | x | | | |
| Data acquisition | | | x | x | | |
| Data analysis | | | | x | x | |
| Statistical analysis | | | | | x | |
| Manuscript preparation | x | | | | | x |
| Manuscript editing | | | | | | x |
| Manuscript review | x | x | x | x | x | x |

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