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IN SILICO STUDIES FOR ANTI-BREAST CANCER *Acmella Oleracea* (L.) FLOWERS

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ABSTRACT
The study of the efficacy of *Acmella oleracea* (L.) flowers on breast cancer is still in its early stages. The molecular interaction mechanisms underlying *Acmella oleracea's* anti-breast cancer activity will be elucidated using in-silico analysis. For this study, seventeen bioactive compounds were used: spilanthol, alpha- and beta-amyrin ester, stigmasterol, beta-sitosterol, alpha-1-sitosterol, 3-acetylaleuritic acid, scopoletin, vanillic acid, trans-ferulic, (72,9E)-2-oxo-undeca-7,9-dienyl 3-methylbut-2-enoate, beta-caryophyllene, beta-pinene, myrcene, caryophyllene oxide, and limone Canonical smiles were obtained from PubChem and inserted into the PASS server to determine biological activity. Several compounds were docked with protein targets, such as ESR1, MAP2K2, and PGR. We used Pyrx 0.8 software for anchoring molecular interaction and Discovery Studio software to visualize the complex binding. In terms of Antineoplastic, apoptosis agonist, caspase-3, caspase-8 stimulant, ovulation inhibitor, steroid synthesis inhibitor, and TP53 expression enhancer, all the compounds tested positive for anticancer activity. According to Swiss ADME and protox analysis, *Acmella oleracea* flowers have the potential to modulate apoptosis and cell growth. More research is required to confirm the role of *Acmella oleracea* bioactive compounds in developing target cancers. The study reveals that Acmella oleracea has numerous bioactive chemicals advantageous for cancer therapy by inducing apoptosis through interaction with ESR1, MAPK2, and PGR protein.

Keywords: *Acmella oleracea*; Anti-cancer; Breast cancer; Bioactive compounds

1. INTRODUCTION

Breast cancer is one of the most malignant cancers in women, which is increasing rapidly in about women (Johnson et al., 2018). In terms of mortality rates, cancer is only surpassed by cardiovascular disease. In this decade prevalence of breast cancer has at least 6. 6 million cases worldwide (Perdana Istyastono, 2015). Based on the newest updated data from the Global Burden of Cancer Study, in 2020, there were 354,243 new cancer cases in Indonesia, resulting in 197,894 deaths. Furthermore, Global Cancer Observatory has predicted that approximately 192,803 Indonesian women are diagnosed with breast cancer yearly. It is widely believed that the rising number of cancer patients in Indonesia is at least partially attributable to the high-dose chemotherapy and other cancer treatments that have become standard practice. Therefore, it can be helpful to create new strategies for approaching breast cancer (Vasan et al., 2019). Using bioactive compounds from natural sources in the alleys as a source of new drugs from wild plants may be the best alternative in cancer therapy. Traditional medicine has long been believed to increase the body's resistance to disease attacks (Wang et al., 2022).

Cancer of the breast typically develops when cells in the breast divide abnormally and metastasize to neighboring tissues (Perdana Istyastono, 2015). Aside from estrogen and estrogen receptors, other factors have been linked to an increased risk of developing breast cancer. This

hormone, estrogen, plays a crucial role in female reproduction. However, both menopausal hormone therapy with estrogen and high levels of endogenous estrogen are associated with an increased risk of breast cancer (Khan et al., 2022). Breast epithelial cells respond to estrogen by increasing the number of estrogen receptors, increasing the rate at which genes are transcribed. Breast cancer can develop when estrogen levels in women are artificially boosted through hyperproliferation (Khan et al., 2022).

Acmella, which belongs to the Asteraceae family, consists of 30 species and nine intraspecific taxa (Peretti et al., 2021; Sharma & Arumugam, 2021). *Acmella oleracea* is one of the most prominent members of the genus *Acmella* and has been regarded as a traditional medicine in various regions of the world, including Asia, Africa, and parts of the Americas, for generations (Sharma & Arumugam, 2021). From previous studies, every aspect of a plant, including its leaves, stems, and fruits, has been shown to alleviate various ailments and diseases. In folk medicine, inflorescences, flowers, and leaves of the *Acmella* genus are used to treat horticulture, aquaculture, insecticides, and spices in various traditional dishes (Benelli et al., 2019; Greger, 2016).

Extensive phytochemical studies on *Acmella oleracea* have been reported previously. It forms different groups of compounds. It has been found to contain many bioactive compounds important for therapeutic applications, including spilanthol, amyrin ester, stigmasterol, miriclic alcohol glycosides, sitosterol, saponins, and triterpenes. Phytochemical research has led to the discovery of alkyl amides such as 3-acetylaleuritolic acid, beta-sitosterol, scopoletin, vanillic acid, trans ferulic acid, and transisoferulic acid (Peretti et al., 2021; Sharma & Arumugam, 2021; Benelli et al., 2019; Greger, 2016; Lalthanpuii et al., 2018). The most representative compounds of this class are the alkyl amides, especially spilanthol ((2E,6Z,8E)-N-isobutyl 2,6,8-decatrienamide), also known as affinin (Sharma & Arumugam, 2021). This molecule is known for its pharmacological properties. In vitro studies have proven the bioactive compound Acmella to have anti-inflammatory and antimicrobial (Lalthanpuii et al., 2018), anesthetic (Kang et al., 2016), antipyretic, antioxidant, insecticidal, antiseptic, immune stimulation anti-obesity, and anticancer effects. Phytosterols such as -sitosterol, stigmasterol, and campesterol are well-known compounds beneficial in treating cardiovascular disease, colon cancer, and breast cancer (Suryani, 2018).

Research shows that A*cmella oleracea* is a promising source of therapeutic agents in preventing cancer growth and DNA damage (Lalthanpuii et al., 2018). The extract of *Acmella oleracea* could reduce oxidative stress and inflammatory targets related to the cancer pathway. Which then inducible nitric oxide synthase (iNOS), transcription factors of the nuclear factor-κB family (NF-κB), cyclooxygenase-2 (COX-2), and mitogen-activated protein kinase (MAPK) signaling pathways(Rahim et al., 2021) The previous study by Lalthanpuii et al., (2018). found that the methanol extract of the plant extract is most potent on the lymphoma (Dalston'slymphoma ascites) cells with an IC50 of 147.547 µg/ml, while it does not affect lung carcinoma (V79).

The most prevalent breast cancer treatments are surgery, chemotherapy, hormone therapy, and immune therapy. However, this method has numerous drawbacks. For instance, although chemotherapy kills cancer cells rapidly, it negatively affects the body. They increase hyper-proliferation in normal cells such as hair follicles, bone marrow, and gastrointestinal tract cells. This results in typical chemotherapy side effects such as hair and skin loss or skin (Wijaya & Muchtaridi, 2017). Therefore, researchers seek more effective treatments with fewer adverse effects. In this vein, one approach is to investigate the bioactivity of natural plant compounds. This study aims to determine the in-silico relationship between 17 *Acmella oleracea* compounds and estrogen receptor alpha (ESR1), PGR, and MAP2K2.

2. METHODS

2.1. Biological Activity Analysis with PASS Server

Using the PASS server, we analyzed the biological activity of Acmella oleracea (L.) flowers. The canonical structures of the investigated bioactive compounds were retrieved from PubChem (https://pubchem.ncbi.nih.gov/) and entered into the PASS server. There will be a list of biological activities alongside the values of Pa and Pi. The Pa value in this study was set at pa > 0.5. The Pa value represents the probability of the compound's activity; the more significant the Pa value, the greater the probability of the compound's activity. Compounds with breast cancer-related activity (Pa > 0.5) were chosen for molecular Docking.

2.2. Target Protein Identification

We identified the target proteins of the 17 compounds using the Superpred (https://prediction.charite.de) and Swiss Target Prediction (http://www.targetprediction.ch) web servers. Using STRING (https://string-db.org/), we analyzed the target protein data of each compound to identify the specific breast cancer-related pathways. KEGG pathway analysis results include PD-L1 expression and the PD-1 checkpoint pathway in cancer, the estrogen signaling pathway, the p53 signaling pathway, and breast cancer. Three of the most potent target proteins were obtained: ESR1, PGR, and MAP2K2.

2.3. Modeling and Validation of the 3-dimensional Structure of the Target Protein

Then 3-dimensional structures of the three target proteins were carried out with Swiss webserver modeling (https://swissmodel.expasy.org/) by inserting the FASTA target protein from the NBCI database. The protein model was validated with a saves webserver (https://saves.mbi.ucla.edu/) based on ERRAT (Over Quality Factor and residual graph) assays and PROCEK (Ramachandran plot and residues in disallowed regions) values.

2.4. Molecular Docking

Molecular Docking was performed on 17 compounds exhibiting estrogen-related activity in breast cancer signaling pathways. Selected bio-active 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 ESR1, PGR, and MAP2K2. The proteins were pre-pared to remove water molecule and unwanted ligand using Discovery Studio 16 software. Molecular docking was done using Pyrx 0.8. Ligands docking to ESR1 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 PGR 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 MAP2K22 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.

2.5. Pharmacochemical, Physicochemical and Toxicity Analysis

Utilizing the SwissADME webserver (http://www.swissamde.ch/), the pharmaceutical and physicochemical properties of the investigated bioactive compounds were analyzed. The canonical SMILE compounds are extracted from PubChem and then added to SwissADME. While the toxicity of bioactive compounds is assessed using the Protox web server (https://tox-new.charite.de/protox II/) by inserting Canonical SMILE compounds from PubChem into Protox, with parameters such as carcinogenic, hepatotoxicity, mutagenic, cytotoxicity, and immunotoxicity.

3. RESULTS AND DISCUSSION

3.1. Apoptotic-Related Activity Screening Analyzed

Screening of the PASS online web server showed the biological activity of compounds related to antineoplastic, apoptosis agonist, caspase-3, caspase-8 stimulant, ovulation inhibitor,

steroid synthesis inhibitor, and TP53 expression enhancer (Figure. 1). Compounds with the specified activity were chosen and analyzed further. PASS analysis yielded two values of probability: Pa (to be active) and Pi (to be inactive). The greater the ratio of Pa to Pi, the greater the likelihood of a compound's biological activity. The study employed a cut-off Pa > 0.5 to obtain a probability of greater than 50 percent compound apoptosis activity. *Acmella oleracea* (L.) flowers contain 12 compounds in the cancer regulation pathway. Scopoletin displayed the most excellent TP53 expression enhancer (Pa = 0.941) compared to other compounds. Beta-Amyrin showed the highest levels of Apoptosis agonist (0.923), antineoplastic (0.916), Caspase 3 (Pa= 0.976, and caspase eight stimulant (Pa = 0.974, among others). Stigmasterol showed the highest Ovulation inhibitor activity (Pa = 0.68) and steroid synthesis inhibitor (Pa= 0.52), among others. Our current result discovered that the bioactive *Acmella oleracea* (L.) flowers might be a promising candidate for regulating cancer associated with apoptosis dan estrogen pathways based on the database server

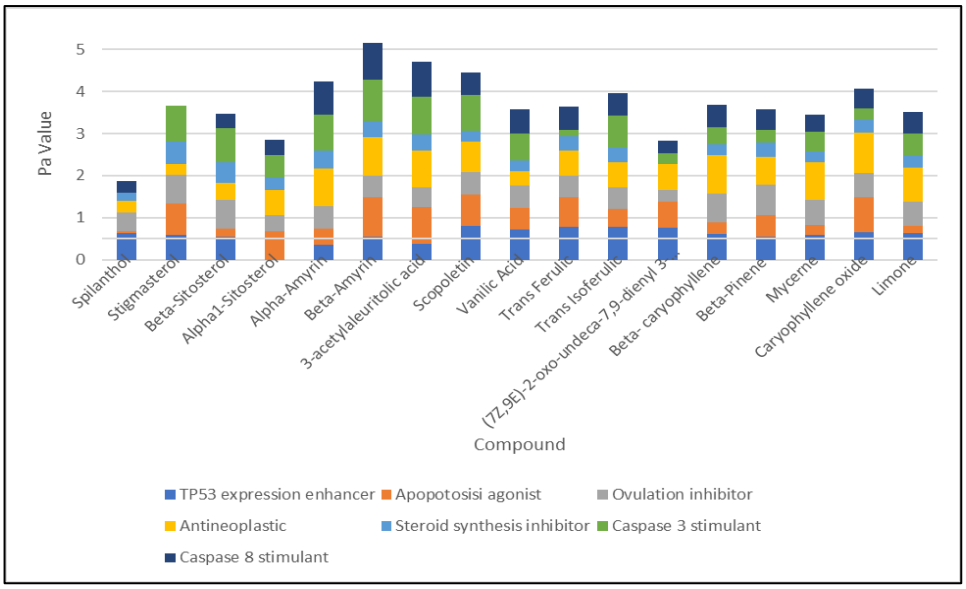

Figure 1. Apoptotic-related activity screening analyzed

3.2. Macromolecular Modelling and Validation

ESR1, MAP2K2, and PGR have been utilized as primary therapeutic targets in the treatment of Breast Cancer. The target protein receptor's 3-D structure was retrieved from the SWISS model with the highest seq-identity (Figure 2). The accuracy of the protein modeling was predicted using the Ramachandran plot. The Ramachandran plot indicates the stereochemical property of the structural structure. The PROCHECK compares the overall model geometry to the residues' geometry and calculates an expected model's stereochemical quality. As input, the PROCHECK tool requires a model protein file and generates the Ramachandran plot. The analysis of Residues in disallowed Ramachandran plots revealed minimal model protein residues. The protein model is more accurate for the fewer residues in disallowed regions. According to Table 1, the PGR protein model has a negligible number of residues (0%).

The ERRAT contains a database of highly refined protein structures and graphs the position's value. This diagram is based on the refined structure database's compilation of nonbonded interaction statistics between different atom types. The result of the ERRAT server is a graph depicting the relationship between residues and error values (Table 1). This input structure's overall quality score of 86.55% for ESR1 and 92% for MAP2K2 is good. However, the design of PGR displays excellent resolution (99%). If the input structure has the adequate resolution, its quality score should exceed 95%.

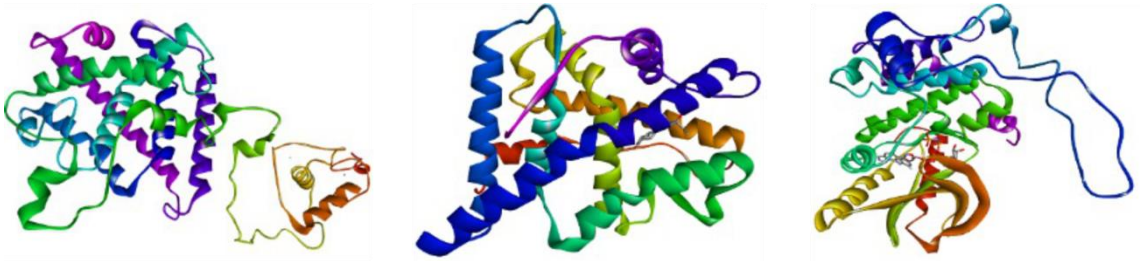

Figure 2. Macromolecular ESR, MAP2K2, and PGR

Table 1. Macromolecular Model Validation Test Results

Macromolecular	Residues in disallowed regions (PROCHECK)	Quality Factor (ERRAT)
ESR1	0.6%	86.550%
MAP2K2	1%	92%
PGR	0%	99.58%

3.3. Analysis of Molecular Docking

The best molecules for anti-breast cancer are obtained by looking at the binding energy value of docking results. The best binding energy is the most minor, meaning that the energy required to bind to the target protein is smaller so that it is more effective in binding to receptors (Islam et al., 2020). Beta-Amyrin exhibited the highest binding affinity against ESR1 (-8.5 kcal/mol) and MAP2K2 (-9.5 kcal/mol), as determined by the molecular docking analysis. In contrast, stigmasterol exhibited the highest binding affinity against PGR (-8.4 kcal/mol) (Table 2).

Table 2. The Binding Affinity of 17 Selected Compounds of Acmella oleracea.

Ligand	Binding Affinity		
	ESR1	MAP2K2	PGR
Control			
Venetoclax	**-8.9**	**-12.6**	**-9.2**
N-Alkyl amide			
Spilanthol	**-5.6**	**-5.9**	**-5.9**
Stigmasterol	**-7.3**	**-8.5**	**-8.4**
Beta-Sitosterol	**-7.0**	**-8.2**	**-7.0**
Alpha1-Sitosterol	**-7.0**	**-8.1**	**-7.6**
Alpha-Amyrin	**-8.6**	**-9.3**	**-7.0**
Beta-Amyrin	**-8.4**	**-9.5**	**-7.6**
3-acetyl aleuritic acid	**-7.7**	**-8.3**	**-7.4**
Scopoletin	**-6.5**	**-5.9**	**-6.6**
Vanillic Acid	**-5.3**	**-6.3**	**-4.7**
Trans Ferulic	**-5.1**	**-6.9**	**-5.8**
Trans Isoferulic	**-5.5**	**-8.6**	**-6.4**
Acmelonate			
(essential9E)-2-oxo-undeca-7,9-diene 3-methylbut-2-enoat)	**-4.7**	**-5.5**	**-5.0**
Triterpene			
Beta- caryophyllene	**-5.7**	**-5.8**	**-7.7**
Beta-Pinene	**-4.8**	**-6.4**	**-5.6**
Myrcene	**-4.7**	**-4.8**	**-4.7**
Caryophyllene oxide	**-5.6**	**0**	**-7.8**
Limone	**-5.7**	**-6.1**	**-5.4**

3.3.1. Molecular Docking of Bioactive Substances of Interest to the ESR1 Protein

Based on molecular docking analysis (Figure 3), the binding energy of the complex of venetoclax and ESR1 protein was -8.9 kcal/mol, involving multiple types of interaction via amino acid residues (Table 2). Seven conventional hydrogen bond interactions and seven hydrophobic ones were identified. These interactions involved the amino acid residues CYS381, PHE461,

LEU544, MET543, SER464, and MET522. These residues were then identified as the essential amino acid residues in the venetoclax-induced production of the ESR1 protein. However, the binding of stigmasterol to ESR1 protein resulted in the formation of ten hydrophobic residues, which included Leu699, PRO696, ARG766, MET692, TRP765, and HIS770. Alpha-Amyrin, Beta-Amyrin, and 3-acetyl aleuritic acid interactions with ESR1 exhibited the lowest binding affinity compared to other bioactive compounds and the closest binding energy to venetoclax, -8.6, -8.4, and -7.6 kcal/mol, respectively. The three compounds have been shown to have bioactive cancers that can play a role in regulating estrogen and breast cancer. They include TP53 expression enhancer, Apoptosis agonist, Ovulation inhibitor, Antineoplastic, Steroid synthesis inhibitor, Caspase 3 stimulant, and Caspase 8, which plays a crucial role in the final step of apoptosis and cell growth (Bellumori et al., 2022; Sharma & Arumugam, 2021). Alpha-Amyrin and ESR1 formed eight hydrophobic alkyl interactions. Beta-Amyrin, with higher binding energy, exhibited eleven hydrophobic interactions with ESR1. With the ESR1 protein, 3-acetylaleuritic acid formed one hydrogen bond and eleven hydrophobic interactions.

As a member of the nuclear receptors family, the estrogen receptor (ESR1) typically functions as a ligand-activated transcription factor. The binding of ligands induces conformational changes in receptors, leading to translocations into the nucleus and transcriptional activation of some target genes (Poirier et al., 2022). ESR1 affects the ligand-binding domains of these proteins. ESR1 is a crucial mechanism for developing endocrine resistance in breast cancer. Numerous amino acid residues, including Tyrosine, Serionin, Aspargin, Glycine, Glutamine, and Aspartic Acid, serve as activation sites for ESR ligands (Robinson et al., 2013). Through these amino acids, the catalytic activity of this protein activated mutation Estrogen. The interaction may alter the conformation of the ESR1 protein, causing it to become mature or cleaved. However, the study did not identify any residues involved in the complex interaction of investigated bioactive compounds with ESR1 protein comparable to the interaction between venetoclax and ESR1 complexes or the phytoconstituents of *Acmella oleracea* Flowers.

3.3.2. Molecular Docking of Examined Bioactive Substances with the MAP2K2 Protein

Figure. 4 depicts the molecular docking results of investigated bioactive compounds and venetoclax against the MAP2K2 protein. The interaction between venetoclax and MAP2K2 protein resulted in four hydrogen bonds (2 Conventional Hydrogen Bonds via LYS101 and SER154, Conventional Hydrogen Bond-Halogen via TYR233, 3 Carbon Hydrogen Bonds via ASP194, MET233, GLY81, and 1 Pi-Donor Hydrogen Bon via SER232). This complex formed 13 hydrophobic interactions via leucine, valine, isoleucine, alanine, methionine, and tyrosine. It was hypothesized that these amino acid residues were crucial for inhibiting MAP2K2 by venetoclax. This complex had a binding energy of -12.6 kcal/mol. An essential residue of LEU201 was involved in multiple interactions, including two hydrophobic interactions between MAP2K2 against stigmasterol, three hydrophobic interactions between MAP2K2 and Alpha-sitosterol, four hydrophobic interactions between MAP2K2 and beta-sitosterol, two hydrophobic interactions between MAP2K2 and scopoletin, and one hydrophobic interaction between MAP2K2 and vanillic acid.

A residue VAL86, as the essential residue of the venetoclax-MAP2K2 complex, is also present in the complexes of spilanthol-MAP2K2 (hydrophobic), stigmasterol-MAP2K2 (hydrophobic), Alpha and beta Amyrin-MAP2K2 (Hydrophobic), scopoletin-MAP2K2 (hydrophobic), Vanillic Acid-MAP2K2 (hydrophobic), trans iso (hydrophobic). The final essential residue, ALA99, was identified as a hydrophobic interaction in the complex of Alpha sitosterol-MAP2K2 protein, Beta sitosterol-MAP2K2 protein, scopoletin-MAP2K2 protein, and vanillic acid-MAP2K2 protein. The involvement of several key residues in the complex of bioactive compounds against MAP2K2 protein was hypothesized to inhibit MAP2K2 protein as venetoclax by interfering with its activity. The research revealed that beta-Amyrin (-9.5 kcal/mol),

Alpha Amyrin (-9.3 kcal/mol), trans isoferulic (-8.6), and stigmasterol (-8.5) have the lowest binding affinity than venetoclax (-12.6 kcal/mol) (Table 1). The interaction may alter the conformation of ESR1 so that it is either mature or cleaved by MAP2K2. Comparable to the venetoclax and MAP2K2 complexes or the compound of *Acmella oleracea* flowers. However, neither beta-Amyirin nor alpha-Amyirin contained a residue implicated in the complex interaction of investigated bioactive compounds with MAP2K2 protein.

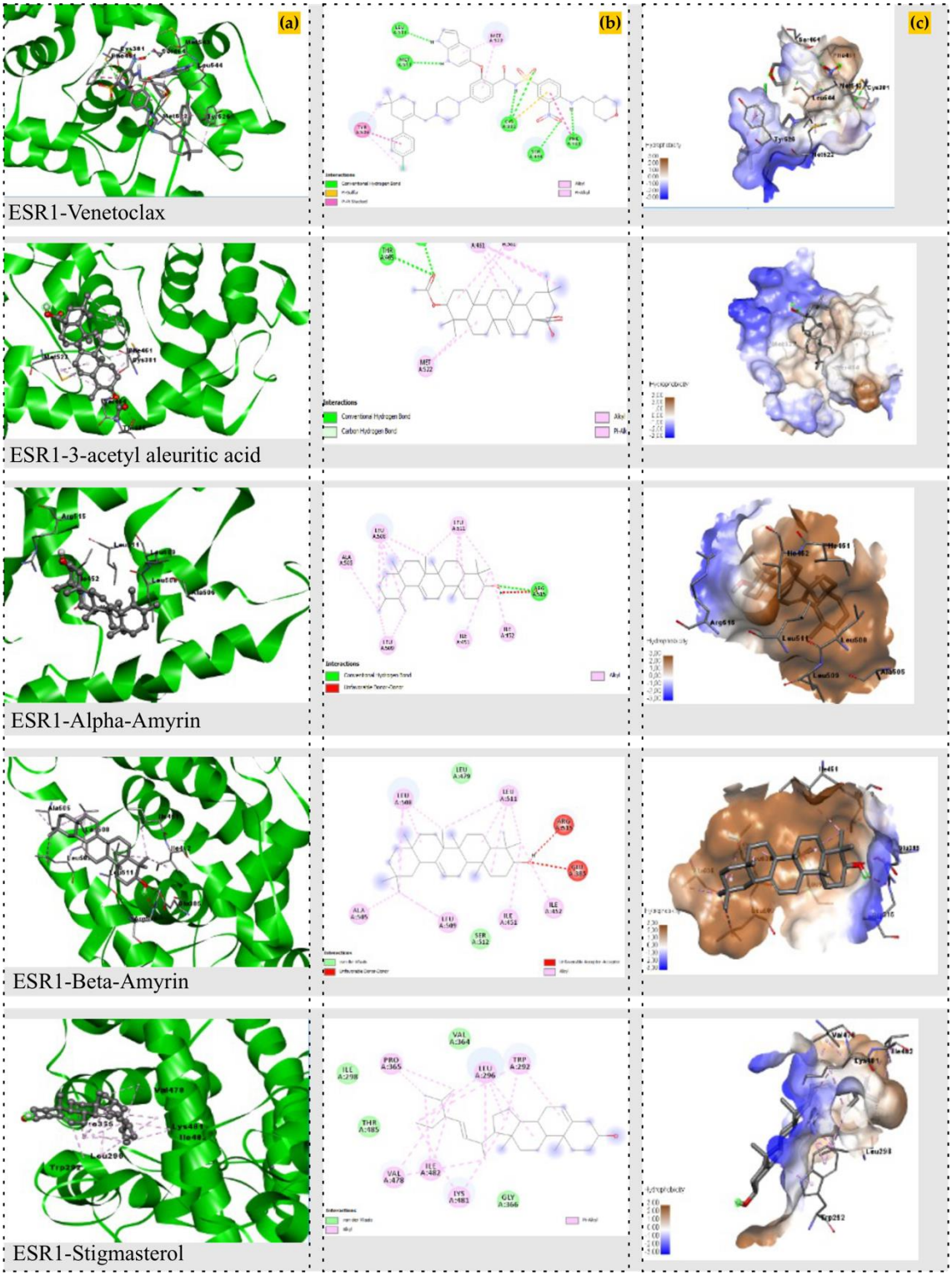

Figure 3. Bioactive compounds interaction against ESR1 protein top 5 lowest binding affinities: (a) the active site of the ligand-protein complex; (b) the complex's two-dimensional structure; (c) its hydrophobicity. The flat green ribbon represented ESR1, whereas the element ball-and-stick represented a bioactive compound

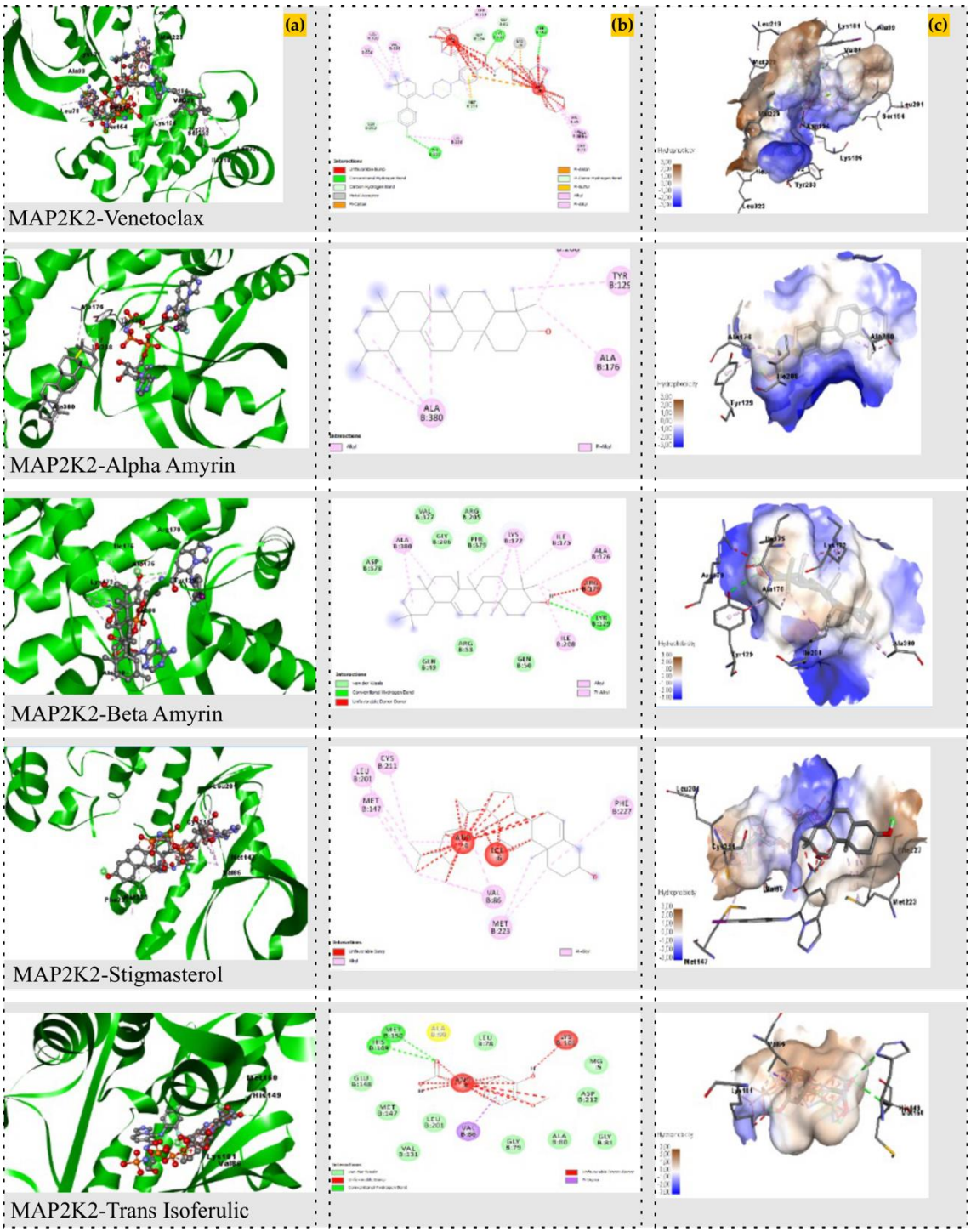

Figure 4. Bioactive compounds interaction against MAP2K2 protein top 5 lowest binding affinities: (a) the active site of the ligand-protein complex; (b) the complex's two-dimensional structure; (c) its hydrophobicity. The flat green ribbon represented MAP2K2, whereas the element ball-and-stick represented a bioactive compound.

3.3.3. Molecular Docking of Bioactive Substances of Interest to the PGR Protein

Progesterone has two types of progesterone receptors, progesterone receptor A (PRA) and progesterone receptor B (PRB). Both receptors cause the transcription of certain genes that have a specific expression of estrogen. It is suspected that PRA inhibits the effects of PRB, and the inhibition extends to the point of affecting estrogen (The impact of PRA inhibition on steroid sex is utilized to counteract the effects of endometrial proliferation by estrogen on the use of Thyroid Stimulating Hormone (TSH) (Kolatorova, et al. 2022). As a background, molecular docking study (Table 2), the binding energy of the venetoclax and PGR protein complex was -9.2 kcal/mol,

involving many types of interaction via amino acid residues (Table 4). There was one carbon-hydrogen bond interaction via SER728 and LYS731, two electrostatic (Pi-cation) interactions via ARG728 and LYS731, and seven hydrophobic interactions via SER728 and LYS731 (1 Pi-P stacked via TYR700, Alkyl via ARG724, and 7 Pi-Alkyl via ARG724, LEU727, ILE699, LYS731, and TYR700).

However, the binding affinity of complex stigmasterol-PGR to PGR protein was 84 percent lower than that of Venetoclax-PGR. Ten hydrophobic (8 alkyl and two Pi-alkyl) residues were produced, including isoleucine, proline, arginine, methionine, tryptophan, and histidine. The compound has been shown to have bioactive cancers that play a role in the regulation of estrogen and breast cancer. They include antineoplastic, apoptosis agonist, caspase-3, caspase-8 stimulant, ovulation inhibitor, steroid synthesis inhibitor, and TP53 expression enhancer, which plays a crucial role in the final step of apoptosis and cell growth (Pu et al., 2022; Siao et al., 2015). This study investigated the interaction mechanism between venetoclax and PGR protein. Four hydrogen bonds, an electrostatic interaction, and eleven hydrophobic contacts bind Venetoclax to PGR. The binding energy of residues proline, glycine, glutamate, alanine, and others were -8.8 kcal/mol. However, the distinctiveness of the three activation sites is not present in venetoclax, the control molecule. Hydrophobic interactions between the bioactive chemicals Beta-pinene and Beta-caryophyllene allowed us to locate the location (binding affinity -5.6 and -7.7). The organic component of the terpene class is found in the essential oil of numerous plants, alpha-Pinene, and beta-pinene inhibiting tumor necrosis factor (TNF)- α-induced invasiveness of MDA-MB-231 cells (De Albuquerque Barros & Henrique Morgon, 2022; Kang et al., 2016; Zang et al., 2022). Analysis revealed that α-pinene dose-dependently inhibited TNF-α-induced matrix metalloproteinase-9 gene promoter activation and mRNA expression.

Stigmasterol has the lowest binding energy (-8.4 kcal/mol), followed by caryophyllene oxide (-7.8 kcal/mol) and Alpha sitosterol and beta Amyrin (-7.6 kcal/mol). Previous research examined the impact of stigmasterol on proapoptotic signals, mitochondrial activity, formation of reactive oxygen species, and cytosolic and mitochondrial calcium levels in human ovarian cancer cells (Bae et al., 2020; Wang et al., 2022). The visualization of the interaction of molecular Docking is depicted in Figure 5.

3.4. Analysis of Pharmacochemical, Physicochemical and Toxicity

The optimal physical properties for bioactive substances to be eaten orally include molecular weight (150-500 g/mol), polarity (TPSA between 20-1302), solubility (log S not more excellent 6.0), flexibility (not greater 9.0 rotatable bonds), and lipophilicity (XLOGP3 between -0.7 and + 5.0) (Daina et al., 2017). The optimal criteria range consisted of Spilanthol, Beta-caryophyllene, Beta-Pinene, Myrcene, Caryophyllene oxide, and Limone. In contrast to Limone, which has a TPSA 20, Stigmasterol, Beta-Sitosterol, Alpha1-Sitosterol, Alpha-Amyrin, Beta-Amyrin, and 3-acetylaleuritolic acid all have >XLOGP (Table 3).

The PROTOX Toxicity test is conducted to determine the compound's toxicity. The positive test results show that the phytoconstituent is not mutagenic, so it does not cause carcinogenic for body cells. In addition, Table 4 shows that most of the bioactive compounds of *Acmella oleracea* are predicted not to cause toxicity. These parameters, including hepatotoxicity, carcinogenicity, mutagenicity, cytotoxicity, and are not extreme in Estrogen Receptor Alpha (ER), Estrogen Receptor Ligand Binding Domain (ER-LBD) effect, and become tumor phosphoprotein suppressor p53. For oral toxicity in rodents (LD50) of bioactive compounds, in silico tests and classification of compound toxicity based on the Globally Harmonized System (GHS) were carried out using the Protox online tool. By considering the prediction of toxicity effects, *Acmella oleracea* extract will be safe if it is consumed with a range of <400mg/kg body.

Table 3. Pharmacochemical and Physicochemical Analysis

Compound	LIPO	SIZE	POLAR	INSOLU	INSATU	FLEX	H-Bond Acceptor	H-Bond Donor
Spilanthol	3.57	221.34	29.10	-2.93	0.5	8	1	1
Stigmasterol	8.56	412.69	20.23	-7.46	0.86	5	1	1
Beta-Sitosterol	9.34	414.71	20.23	-7.9	0.93	6	1	1
Alpha1-Sitosterol	9.03	426.72	20.23	-7.84	0.87	5	1	1
Alpha-Amyrin	9.01	426.72	20.23	-8.16	0.93	0	1	1
Beta-Amyrin	9.15	426.72	20.23	-8.25	0.93	0	1	1
3-acetylaleuritolic acid	8.40	498.74	63.60	-8.03	0.88	3	4	1
Scopoletin	1.53	192.17	59.67	-2.46	0.1	1	4	1
Vanilic Acid	1.43	168.15	66.76	-2.02	0.12	2	4	2
Trans Ferulic	1.51	194.18	66.76	-2.52	0.1	3	4	2
Trans Isoferulic	1.51	194.18	66.76	-2.11	0.1	3	4	2
(7Z,9E)-2-oxo-undeca-7,9-dienyl 3-methylbut-2-enoat)	4.13	264.36	43.37	-3.42	0.5	10	3	0
Beta-caryophyllene	4.38	204.35	68.78	-3.87	0.73	0	0	0
Beta-Pinene	4.16	136.23	45.22	-3.31	0.8	0	0	0
Myrcene	4.17	136.23	48.76	-3.05	0.4	4	0	0
Caryophyllene oxide	3.56	220.35	68.27	-3.45	0.87	0	1	0
Limone	4.57	136.23	0	-3.5	0.6	1	0	0

Table 4. Analysis Toxicity

Compound	Hepato toxicity	Carcino genicity	Immuno toxicity	Muta genicity	Cyto toxicity	ER*	ER-LBD**	p53***
Spilanthol	X	X	X	X	X	X	X	X
Stigmasterol	X	X	√	X	X	X	X	X
Beta-Sitosterol	X	X	√	X	X	X	X	X
Alpha1-Sitosterol	X	X	√	X	X	X	X	X
Alpha-Amyrin	X	X	√	X	X	X	X	X
Beta-Amyrin	X	X	√	X	X	X	X	X
3-acetylaleuritolic acid	X	X	√	X	X	X	X	X
Scopoletin	X	√	√	X	X	X	X	X
Vanillic Acid	X	X	X	X	X	X	X	X
Trans Ferulic	X	X	√	X	X	X	X	X
Trans Isoferulic	X	X	√	X	X	X	X	X
(7Z,9E)-2-oxo-undeca-7,9-dienyl 3-methylbut-2-enoat)	X	X	X	X	X	X	X	X
Beta-caryophyllene	X	X	√	X	X	X	X	X
Beta-Pinene	X	X	X	X	X	X	X	X
Mycerne	X	X	X	X	X	X	X	X
Caryophyllene oxide	X	X	√	X	X	X	X	X
Limone	X	X	X	X	X	X	X	X

*ER = Estrogen Receptor Alpha; **ERLBD = Estrogen Receptor Ligand Binding Domain; ***P53 = Phosphoprotein (Tumor Suppressor)

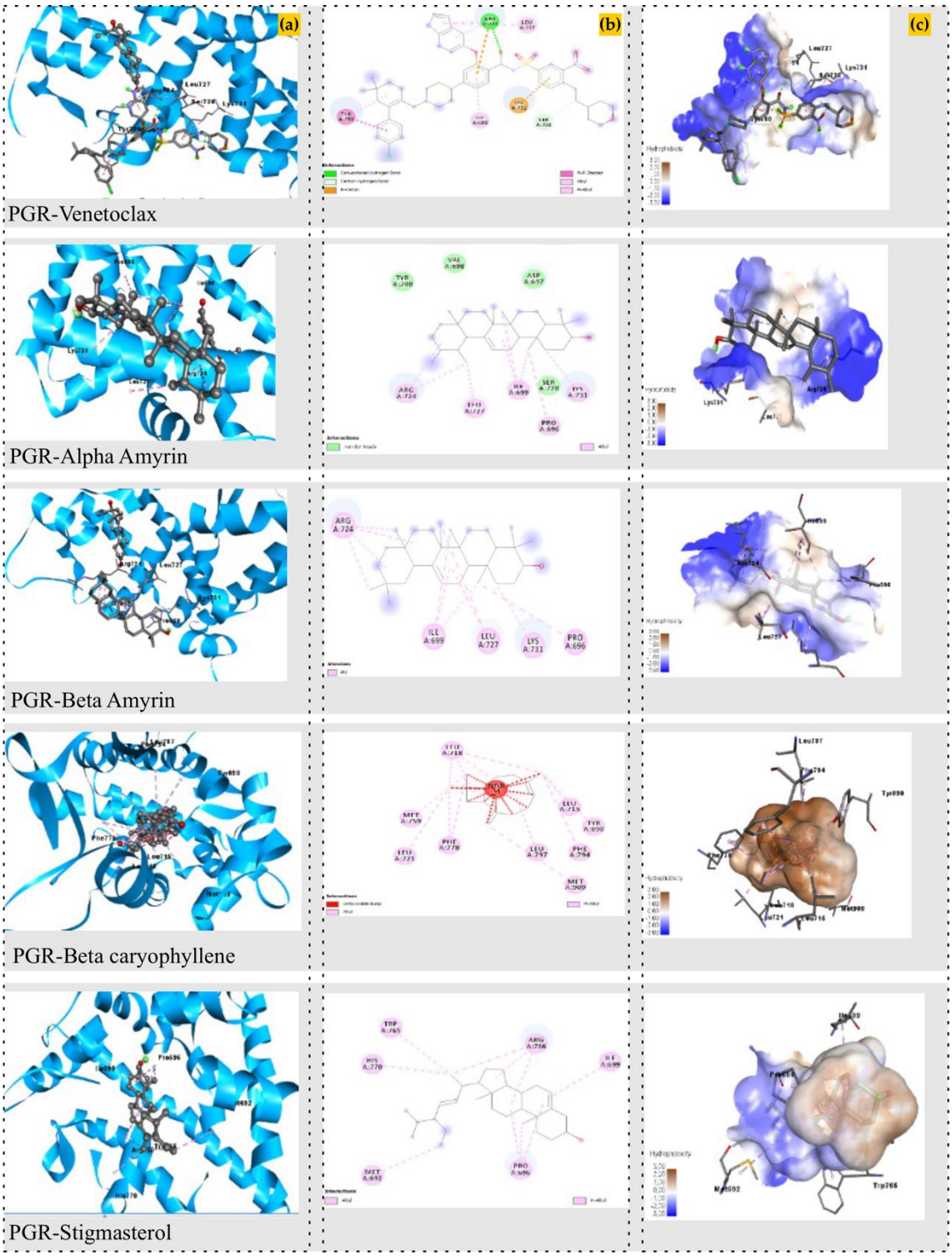

Figure 5. Top 5 lowest binding affinity interactions of the bioactive compound against PGR protein: (a) the active site of the ligand-protein complex; (b) the complex's two-dimensional structure; (c) its hydrophobicity. The flat green ribbon was PGR, whereas the ball-and-stick part was a bioactive chemical

4. CONCLUSION

In silico analysis revealed the molecular mechanisms behind *Acmella oleracea* (L.) Flowers as an anti-breast cancer agent. This study showed that bioactive chemicals derived from *Acmella oleracea* (L.) flowers may have the ability to influence apoptosis and cell proliferation. The amyrins (natural triterpene compounds), such as alpha amyrin and beta amyrin from the lignans group, were expected to be potent ESR1, MAP2K2, and PGR inhibitors. In contrast, stigmasterol, a flavonoid, was projected to be the most potent PGR inhibitor. The study reveals that *Acmella oleracea* has numerous bioactive chemicals advantageous for cancer therapy by inducing

apoptosis through interaction with ESR1, MAPK2, and PGR protein. Bioactive chemicals from *Acmella oleracea* (L.) flowers may limit cancer cell development and trigger apoptosis by interfering with antineoplastic, apoptosis agonist, caspase-3, and caspase-8 stimulant, ovulation inhibitor, steroid synthesis inhibitor, and TP53 expression enhancer. Further, to validate it, additional research is necessary.

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6. CONFLICT OF INTEREST

All authors declare no conflict of interest.

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