The Anti Breast Cancer Activity of *Ficus deltoidea* Jack : A Computational Analysis of Its Biological Activities

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Abstract: Breast cancer is the most common diagnosed cancer worldwide with total 2.3 million cases. Impaired apoptosis plays a central role in the development of breast cancer and can reduces the efficacy of traditional cytotoxic therapies. *Ficus deltoidea* Jack containing many beneficial chemical compounds especially vitexin, isovitexin, moretenol, luteolin, vicenin-2 and orientin which established anticancer effect. The aim of study was to investigate breast cancer activity of *Ficus deltoidea Jack* six bioactive compounds specifically on apoptosis using computational study. PASS (Prediction of Activity Spectra for Substances) was used to analyze the bioactive compounds. The analysis continues with an insilico study on protein Bcl-2 and Caspase 3. From molecular docking moretenol had highest docking score on Bcl 2 (-7,99 kcal/mol) and caspase 3 (-7,6 kcal/mol) compared to vitexin, isovitexin, luteolin, vicenin-2 and orientin. Based on PASS analysis, isovitexin, vitexin, orientin, luteolin and vicenin-2 could enhance TP53 gene expression which encodes the p53 tumor suppressor protein that is pivotal in apoptosis, while moretenol had activity as caspase 3 stimulant. These results can be a reference for further study whether in vitro or in vivo.

1 INTRODUCTION

Female breast cancer is the most common diagnosed cancer worldwide in 2020, with an estimated 2.3 million new cases or 11.7% from total 18,1 million cases, followed by lung, colorectal, prostate, and stomach cancers. In 2040, the breast cancer cases are predicted will be increased into 3 million cases per year or grow 40% from 2020 data. Apoptosis is one of important target in breast cancer. In cancer cell progression, apoptosis is decreasing (Pfeffer & Singh, 2018). Protein caspase 3 is an effector protein in apoptosis, while Bcl-2 is an anti-apoptosis protein which inhibit this process(Nguyen et al., 2021).

Current cancer treatments are surgery, chemotherapy, and radiation These three treatments involve a rather invasive approach and often causes side effects. Serious side effects can reduce a patient's quality of life, lead to non-compliance to chemotherapy, and lead to drug resistance (Akhlaghi et al., 2020). Some cancer patients use alternative therapy such as using herbal medicine to treat the cancer. Patients try to discover and utilize natural-derived anti-cancer drugs because it believes has fewer side effects and more affordable.

The utilization of herbal-based therapy is on the rise as a valuable and efficient approach for cancer treatment. This is due to the fact that herbal plants offer a vast array of phytochemical compounds, which serve as crucial elements in the development

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and exploration of novel drugs. *Ficus deltoidea* Jack a plant from Indonesia is empirically used by local community as alternative for cancer therapy. This plant leaves extract mostly containing flavan-3ol monomer, c-linked flavone glycosides like vitexin and isovitexin, proantocyanidins and also apigenin and luteolin (Omar et al., 2011). Flavonoid is phenolic compound which is widely exist in plants and significantly show anti apoptosis activity based on in vitro or in vivo study(Hu et al., 2019). This study, therefore is to investigate breast cancer activity of *Ficus deltoidea Jack* six bioactive compounds specifically on apoptosis using several tools, Autodock Vina, and PASS (Prediction of Activity Spectra for Substances).

2 MATERIAL AND METHODS

2.1 The Biological Activity Prediction Using PASS

Six compounds from *Ficus deltoidea* Jack were analyzed for their biological activity using the Prediction of Activity Spectra for Substances (PASS) which can be accessed at http://www.pharmaexpert.ru/passonline. Canonical SMILES of each compound are inputted on the website and a list of the biological activity of the compound will appear based on the existing database on PASS. The activity list is accompanied by Pa (Probably active) and Pi (Probably in active) values. The Pa value is closer to 1 the better and a good Pi value is closer to 0.

2.2 Molecular Docking

The protein used are Bcl-2 (PDB ID : 2w3l) and Caspase 3 (PDB ID : 3kjf) were accessed from the RCSB Protein Data Bank (https://www.rcsb.org/). Unneeded water molecules, ligands, and chains are removed. The software used is Autodock Vina 1.5.7 grid box with dimensions of x= 60, y=60, and z = 60 and center x= 49.701 Å, y= 33.626 Å, z= -13.207 Å for Bcl-2 and x= 40, y=40, and z = 40 and center x= -27.789 Å, y= -6.843 Å, z= 16.216 Å for Caspase 3. After docking, visualization was performed using Discovery Studio.

3 RESULT AND DISCUSSIONS

Six chemical compounds contained in *Ficus deltoidea* from database were docked with protein Bcl-2 and Caspase 3, two proteins which are important in apoptosis pathway. Moretenol is a pentacyclic terpenoid belonging to the moretenane-type triterpenes. Vitexin and isovitexin are glycosides with aglycone belongs to flavone subclass, apigenin (Choi & Kim, 2009). Vicenin-2 is also glycoside which has similar structure like isovitexin, but it hydrogen in 8 position is replaced by Beta-D-glucosyl residue. Glycosides orientin and luteolin have similar structure, but C-glycosyl on 8 positions of luteolin is replaced by beta-D-glucopyranosyl.

From docking study, six compounds can interact with Bcl2 protein through hydrogen binding (table 1). Some compounds bind into similar amino acid residues. Vitexin and luteolin could bind into Ala 108 amino acid residue. Vitexin and vicenin-2 could bind to Val 92 and Glu 95 amino acid residues. Vitexin and orientin could bind to Glu95 amd Asp70. Isovitexin and luteolin could bind to Asn 102. From six compounds only, moretenol and protein Bcl-2 which produce one hydrogen bond in Asp 99 amino acid residues. Eventhought there was only one hydrogen bond, interaction between moretenol and bcl-2 had lowest gibbs energy, -7.99 kcal/mol.

Vitexin, vicenin-2 and isovitexin could bind to caspase 3 through hydrogen bond in Arg 207 amino acid residues. Vitexin, vicenin, luteolin and moretenol could also bind to similar amino acid residues, PHE 250. From 6 compounds, moretenol interaction had lowest gibbs energy. Lower bonding ΔG and Ki values, remarking that compound can be suitable ligand for protein because low delta gibbs energy and inhibition constanta (ki) means interactions occur spontaneously and complexes between protein-ligand are stable. Phenolic compound like flavonoid glycoside can form complexes with protein via covalent or non-covalent interaction through hydrogen, van der waals, electrostatic and hydrophobic bonding. Mainly interaction between phenolic compounds and protein are through hydrophobic interaction and hydrogen binding (Shahidi & Dissanayaka, 2023).

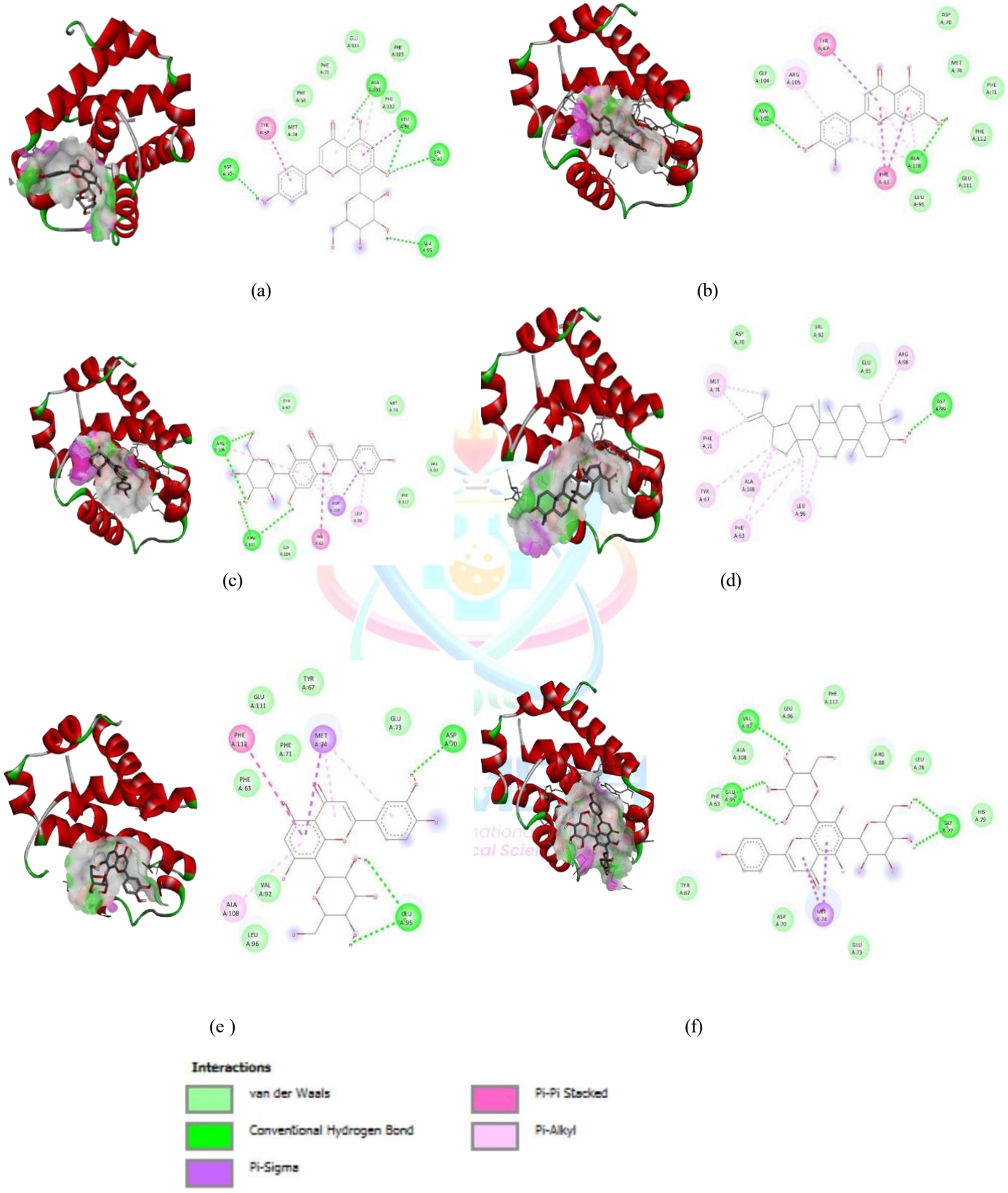

Figure 1: Interaction between Bcl-2 and Vitexin (a), Luteolin (b), Isovitexin(c), Moretenol (d), orientin (e), vicenin-2 (f)

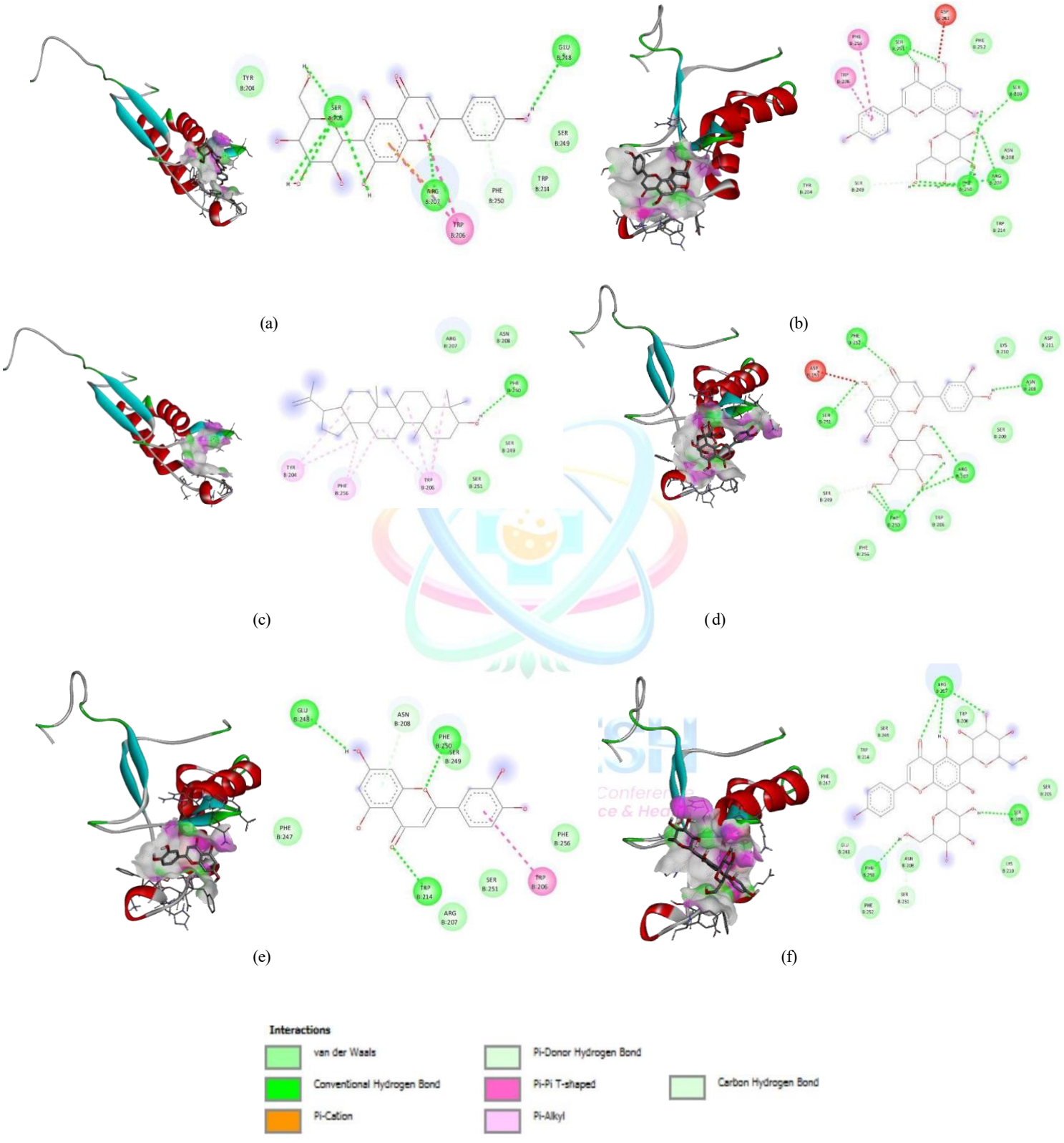

Figure 2: Interaction between Caspase 3 and Isovitexin (a),Vitexin (b), Moretenol (c), Orientin (d), Luteolin(e), Vicenin-2 (f)

Table 1: Molecular Docking Results Six Compounds with Protein Bcl-2 and Caspase 3

Compounds Name	Energy (kcal/mol)		Inhibition Constanta (Ki)		Hydrogen Bond	
	Bcl-2	Caspase 3	Bcl-2	Caspase 3	Bcl-2	Caspase 3
Vitexin	-5.10	-6.62	183.46	13.93	ALA 108, LEU 96, ASP 70, VAL 92, GLU95	ARG 207, SER 209, PHE 250, SER 251
Isovitexin	-5.60	-6,17	78.03	29.97	ARG 105, ASN 102	ARG 207, SER 205, GLU 248,
Moretenol	-7.99	-7,6	1.39	2,7	ASP 99	PHE 250
Luteolin	-5.50	-6,10	92.58	33.73	ALA 108, ASN 102	GLU 248, PHE 250, TRP 214
Orientin	-4.71	-6,46	355.40	18.44	GLU 95, ASP 70	ARG 207, PHE 250, PHE 252, ASN 208
Vicenin 2	-5.12	-5.66	175.24	71.14	VAL 92, GLU 95, GLY 77	ARG 207, SER 209, PHE 250

Besides conducting molecular study, anticancer activity of 6 compounds also had been analysed using computer program called PASS, accessible at (http://www.pharmaexpert.ru/passonline/) was used to predict bioactivity spectra based on chemical structures. This computational method can predict potential in vivo bioactivity for 6 compounds. Furthermore, this method produced a comprehensive list of biological activities along with their Pa and Pi. From the PASS analysis (table 2), activities related to anticancer mechanisms were selected with a cut-off value of >0.8. The selected activities include TP53 expression enhancer, HIF-1a inhibitor, free radical scavenger, cytostatic, anticarcinogenic, apoptotic agonist, and caspase 3 stimulant. Based on PASS analysis, all flavonoid glycoside compounds can enhance TP53 expression, inhibit HIF1A expression, and act as free radical scavenger, cytostatic agent and anticarcinogenic. While moretenol had activity as caspase 3 stimulant and apoptosis agonist. TP53 is a gene which has role in P53 production(Aubrey et al., 2018). P53 has important role in apoptosis, this protein can induce BAX, pro apoptosis protein, and inhibit Bcl-2 pro apoptosis protein(Li & Yuan, 2008)

Table 2: Biological activity related to cancer prediction results analyzed using PASS

Anticancer Activity	Vitexin		Isovitexin		Vicenin-2		Orientin		Luteolin		Moretenol	
	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi
TP53 expression enhancer	0.973	0.003	0,962	0,003	0,951	0,003	0,974	0,003	0,916	0,004	-	-
HIF1A expression inhibitor	0,940	0,004	0,920	0,005	0,915	0,005	0,940	0,004	0,964	0,003	-	-
Free radical scavenger	0,901	0,002	0,845	0,002	0,877	0,002	0,955	0,001	-	-	-	-
Cytostatic	0,869	0,005	0,885	0,004	0,852	0,005	0,875	0,004	-	-	-	-
Anticarcinogenic	0,866	0,003	0,856	0,004	0,831	0,004	0,872	0,003	-	-	-	-
Caspase 3 Stimulant	-	-	-	-	-	-	-	-	-	-	0,969	0,002
Apoptosis agonist	-	-	-	-	-	-	-	-	-	-	0,846	0,005

4 CONCLUSIONS

Based on molecular docking results, moretenol had highest docking score on Bcl 2 (-7,99 kcal/mol) and caspase 3 (-7,6 kcal/mol) compared to vitexin, isovitexin, luteolin, vicenin-2 and orientin. Based on PASS analysis, isovitexin, vitexin, orientin, luteolin and vicenin-2 could enhance TP53 gene expression which encodes the p53 tumor suppressor protein that is pivotal in apoptosis, while moretenol had activity as caspase 3 stimulant. These results can be a reference for further study whether in vitro or in vivo.

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