

Study of Biological Activity of the Genus *Spatholobus* against Breast Cancer in Silico

Dodi Iskandar¹, Nashi Widodo², Warsito³, Masruri^{3*}, Rollando⁴

¹ Agricultural Technology Department, Polytechnic State Pontianak, Pontianak, Indonesia

² Biology Department, Brawijaya University, Malang, Indonesia

³ Chemistry Department, Brawijaya University, Malang, Indonesia

⁴ Pharmacy Department, Ma Chung University, Malang, Indonesia

Abstract: The development of apoptotic agents from natural plants has potential as promising breast cancer treatment candidates. The study on the efficacy of plants of the genus *Spatholobus* on breast cancer is still very limited. This study aims to explain the molecular mechanism that underlies the biological activity of breast anticancer from plants of the genus *Spatholobus* by in silico analysis. The method used has involved four techniques. First, the thirty-three compounds from plants of the genus *Spatholobus* were analyzed using the PASS server to obtain information about compounds that have breast cancer biological activity above 75%. Second, the thirteen selected compounds were evaluated using the STITCH server to determine their interactions with various proteins involved in apoptotic pathways and p53 signaling. Third, the thirteen breast anticancer compounds were re-selected to get pharmacological properties for safe consumption with the SwissADME server. Lastly, the selected nine compounds were further docked with target protein caspase-3 using the PyRx 0.99 tool and visualized with PyMol 2.5.2 and BIOVIA Discovery Studio Visualizer 2.1.1.0.2098. In conclusion, the nine compounds (lupinalbin A, trigraecum, coumestrol, maackiain, medioresinol, isoliquiritigenin, 8-O-methylretusin, biochanin A, and medicarpin) from the genus *Spatholobus* are predicted to have potential as activating agents for the caspase-3 protein and can suppress the growth of breast cancer cells.

Keywords: anticancer, bioactive compound, genus *Spatholobus*, apoptotic.

用計算機研究雞血藤屬抗乳腺癌的生物學活性

摘要：從天然植物中開發凋亡劑具有作為乳腺癌治療候選藥物的潛力。關於雞血藤屬植物對乳腺癌療效的研究仍然非常有限。本研究旨在通過計算機分析來解釋雞血藤屬植物的乳腺癌抗癌生物活性的分子機制。使用的方法涉及四種技術。首先，使用經過服務器分析了來自雞血藤屬植物的33種化合物，以獲得有關具有75%以上乳腺癌生物活性的化合物的信息。其次，使用縫服務器評估了13種選定的化合物，以確定它們與參與凋亡途徑和p53信號傳導的各種蛋白質的相互作用。第三，使用瑞士人ADME服務器重新選擇了13種乳腺癌抗癌化合物以獲得安全食用的藥理特性。最後，使用PyRx0.99工具將選定的九種化合物與目標蛋白半胱天冬酶-3進一步對接，並使用PyMol 2.5.2和百奧維亞探索工作室展示台2.1.1.0.2098進行可視化。總之，來自雞血藤屬的9種化合物（羽扇豆苷一个、黃芪、香豆雌酚、麥肯、中樹脂醇、異甘草素、8鄰甲基維他命、生物鏈素A、和藥草平）預計具有作為半胱天冬酶-3蛋白激活劑的潛力並且可以抑制乳腺癌細胞的生長。

关键词：抗癌，生物活性化合物，鸡血藤属，凋亡。

Received: November 27, 2021 / Revised: December 06, 2021 / Accepted: January 11, 2022 / Published: February 28, 2022

About the authors: Dodi Iskandar, Agricultural Technology Department, Polytechnic State Pontianak, Pontianak, Indonesia; Nashi Widodo, Biology Department, Brawijaya University, Malang, Indonesia; Warsito, Masruri, Chemistry Department, Brawijaya University, Malang, Indonesia; Rollando, Pharmacy Department, Ma Chung University, Malang, Indonesia

Corresponding author Masruri, masruri@ub.ac.id

1. Introduction

The most cancer in the world in 2020 is breast cancer. WHO reports that as many as 2.26 million cases occurred, with the number of deaths 685,000 people [1]. Most anticancer agents used in clinical oncology use the intact apoptotic signaling pathway to cause cancer cell death [2]. Some proteins have been recognized to play a pivotal role in apoptotic. Caspases are a family that adjusts the process of programmed cell death [3]. Among the caspase proteins, caspase-3 activation plays an important role in suppressing the growth of breast cancer cells [4, 5]. Apoptotic is an automatic stage consisting of initiation, locution, and various genes that cause programmed cell death or remove undesired or abnormal cells in organisms and preserve a stable internal environment [6]. Defective apoptotic cancer cells grow and cause harmful mutations [7]. Apoptotic performance can be improved by natural products and supported by various experiments [8]. The natural product encompasses a major part of restorative materials in cancer treatment specifically [9]. One of the natural products with potential anticancer activity is the plant of genus *Spatholobus*. Some papers mentioned that the compounds from this plant could be an anticancer agents [10-12]. There are reported thirty-four species of genus *Spatholobus* globally, and the other five are currently being verified [13]. Based on previous reports, 33 compounds of this genus had been identified (Table 2). The publications of anti-breast cancer biological activity of compounds from genus *Spatholobus* plants are still limited. The initial potential activity study of genus *Spatholobus* compounds is urgently required. It can be carried out computationally and experimentally. The present preliminary study is conducted to predict the potential biological activity of compounds of the genus *Spatholobus* related to breast cancer in silico.

2. Materials and Methods

2.1. Preparation of Compound SMILES

All compound canonical SMILES of genus *Spatholobus* were retrieved from PubChem.

2.2. Biological Activity Analysis by PASS Server

The biological activity relates to apoptotic is at least caspase-3 stimulant, antineoplastic, apoptotic agonist, TP53 expression enhancer, caspase 8 stimulant, and anti-carcinogenic [14]. The canonical SMILES of genus *Spatholobus* was retrieved from PubChem and inserted in PASS server (<http://way2drug.com/PassOnline/>). The biological activity list will emerge along with the Pa (active) and Pi (inactive) values. This procedure set the Pa values at Pa>0.75 [15]. The score reflects the biological potential. The biological activity of the compound increased along with the high Pa score. The compounds

with apoptotic related to the activity (Pa>0.75) are selected and used for further procedure.

2.3. Ligand-Protein Interaction and Network Analysis

The compounds with the highest biological activity are analyzed using STITCH server (<http://stitch.embl.de/>) to evaluate their interactions with proteins.

2.4. Pharmacological Properties Analysis

The properties of active compound pharmacology are scanned using the SwissADME server (<http://www.swissadme.ch>). The canonical SMILES of genus *Spatholobus* was retrieved from PubChem of most active compounds are inserted into it. The data would be listed on the server and further interpreted with the optimum criteria for safe oral consumption (molecular weight 150-500 g/mol; polarity TPSA 20-130 Å²; solubility Log S >-6; rotatable bonds<9; lipophilicity -0.7<XLOGP3<+5.0) [16].

2.5. Molecular Docking and Visualization

This study used the nine best ligands based on the evaluation of the PASS server. The compounds were taken from PubChem and converted into PDB format using OpenBabel 3.1.1. The protein target of this research was caspase-3 (PDB ID: 1GFW) that retrieved from RSCB Protein Data Bank (<https://www.rcsb.org/>). The protein was set to dispel water molecules and undesired ligands using PyMol 2.5.2. The selected chain was A that contained a control ligand. Molecular docking was carried out by of PyRx 0.99 (Table 6). The ligands docking to caspase-3 were managed to center x =37.078, y =33.591, and z =27.010 with dimension (Angstrom) x = 16.793, y = 14.086, and z = 38.856. The complexes of ligand-protein were presented by the PyMol tool. Biovia discovery studio visualizer 2.1.1.0.2098 was used to show the complex's active sites and 2D structures.

3. Results and Discussion

3.1. The Canonical SMILES of Genus *Spatholobus* Compounds

The canonical SMILES of compounds from genus *Spatholobus* plants are listed in Table 1. *Spatholobus* covers four species (*S. suberectus*, *S. parviflorus*, *S. sinensis*, and *S. Dunn*). The compounds of *S. suberectus* are maackiain, medicarpin, trigraecum, sativan, pseudobaptigenin, genistin [17], naringenin, protocatechuic acid ethyl ester, coumestrol, isoliquiritigenin, lupinalbin A, and leonuriside A [18]. *S. parviflorus* consists of medicarpin, 8-o-methylretusin, biochanin A, trans-4-hydroxymellein, cis-4-hydroxymellein, and coumestrol [19]. The compounds of *S. sinensis* include prestegane B, (+)-medioresinol, benzeneethanol, naringenin, blumenol A,

protocatechuic acid ethyl ester, liquiritigenin, protocatechuic acid, and glycyroside [20]. *S. suberectus* Dunn consists of calycosin, pyromucic acid, 1,3,5-benzenetriol, succinic acid, beta-sitosterol, suberectin [21].

3.2. Apoptotic-Related Activity Screening

The Pa value related to apoptotic is presented in Table 2. There are possibly 11 compounds with a Pa value of more than 0.75 caspase-3 stimulants. They include maackiain, medicarpin, trigraecum, genistin, medioresinol, glycyroside, beta-sitosterol, stigmaterol, isoliquiritigenin, leonuride A, and suberectin. The predicted compounds with Pa value of antineoplastic > 0.75 consist of maackiain, medicarpin, genistin, medioresinol, glycyroside, trans & cis-4-hydroxymellein, isoliquiritigenin, lupinalbin A, and leonuride A. The estimated compounds with Pa score of apoptotic agonist higher than 0.75 covers maackiain, medicarpin, trigraecum, genistin, glycyroside, coumestrol, isoliquiritigenin, and leonuride A.

The predicted compounds with Pa value of TP53 expression enhancer higher than 0.75 embrace medicarpin, trigraecum, genistin, medioresinol, naringenin, liquiritigenin, formononetin, daidzein, calycosin, 1,3,5-benzenetriol, biochanin A, trans & cis-4-hydroxymellein, coumestrol, lupinalbin A, leonuride A, and suberectin. The estimated compounds with Pa value more than 0.75 of caspase-8

stimulant only include glycyroside and leonuride A. Ultimately, the predicted compounds with Pa value higher than 0.75 cover genistin, glycyroside, and leonuride A.

The four compounds with the highest Pa value comprise medicarpin, glycyroside, maackiain, stigmaterol, lupinalbin A, leonuride A, medioresinol, 8-O-methylretusin, biochanin A, isoliquiritigenin, coumestrol, genistin, trigraecum, and genistin (Table 3).

3.3. Pathway Analysis and Protein Interaction

The proteins involved in the apoptotic pathway (false discovery rate 1.3×10^{-7}) are caspase-3, Bcl2, FADD, caspase-8, TP53, and ATM. In contrast, the proteins included in the p53 signaling pathway (false discovery rate 9.31×10^{-10}) are caspase-3, caspase-8, TP53, MDM2, ATM, CDKN1A, and CDKN2A. On pathways, isoliquiritigenin and biochanin A probably activates caspase-3 directly with percentage of 90.7% and 70.0%, respectively (Fig. 1 and Fig. 2). Caspase-3 initiates apoptotic DNA fragmentation by proteolytically inactivating DFF45/ICAD (Fig.14) and acts as a vital marker of the cell's entry point into the apoptotic signaling pathway. Caspase-3 has been revealed to cleave and activate numerous effectors, including SREBPs, caspase-6, caspase-7, and caspase-9 [22].

Table 1 SMILES of compounds from genus *Spatholobus* plants

No	Compound	PubChem Cid	SMILES	Ref.
1	Maackiain	91510	<chem>C1C2C(C3=C(O1)C=C(C=C3)O)OC4=CC5=C(C=C24)OCO5</chem>	[17]
2	Medicarpin	336327	<chem>COC1=CC2=C(C=C1)C3COC4=C(C3O2)C=CC(=C4)O</chem>	[17, 19]
3	Trigraecum	14376438	<chem>COC1=C(C=C2C(=C1)C(=O)C=C(O2)C3=CC=CC=C3)O</chem>	[17]
4	Sativan	596401	<chem>COC1=CC(=C(C=C1)C2CC3=C(C=C(C=C3)O)OC2)OC</chem>	[17]
5	Pseudobaptigenin	5281805	<chem>C1OC2=C(O1)C=C(C=C2)C3=COC4=C(C3=O)C=CC(=C4)O</chem>	[17]
6	Genistin	5281377	<chem>C1=CC(=CC=C1C2=COC3=CC(=CC(=C3C2=O)O)OC4C(C(C(C(O4)CO)O)O)O</chem>	[17]
7	Prestegane B	146425	<chem>COC1=C(C=C(C=C1)CC2COC(=O)C2CC3=CC(=C(C=C3)OC)O)O</chem>	[20]
8	(+)-medioresinol	181681	<chem>COC1=CC(=CC(=C1O)OC)C2C3COC(C3CO2)C4=CC(=C(C=C4)O)OC</chem>	[20]
9	Benzeneethanol	6054	<chem>C1=CC=C(C=C1)CCO</chem>	[20]
10	Naringenin	932	<chem>C1C(OC2=CC(=CC(=C2C1=O)O)O)C3=CC=C(C=C3)O</chem>	[20, 21]
11	Blumenol A	5280462	<chem>CC1=CC(=O)CC(C1(C=CC(C)O)O)(C)C</chem>	[20]
12	Protocatechuic acid ethyl ester	77547	<chem>CCOC(=O)C1=CC(=C(C=C1)O)O</chem>	[20, 21]
13	Liquiritigenin	114829	<chem>C1C(OC2=C(C1=O)C=CC(=C2)O)C3=CC=C(C=C3)O</chem>	[20]
14	Protocatechuic acid	72	<chem>C1=CC(=C(C=C1C(=O)O)O)O</chem>	[20]
15	Glycyroside	101939210	<chem>COC1=CC=C(C=C1)C2=COC3=C(C2=O)C=CC(=C3)OC4C(C(C(C(O4)C)O)O)OC5C(C(CO5)(CO)O)O</chem>	[20]
16	Formononetin	5280378	<chem>COC1=CC=C(C=C1)C2=COC3=C(C2=O)C=CC(=C3)O</chem>	[22, 19]
17	Daidzein	5281708	<chem>C1=CC(=CC=C1C2=COC3=C(C2=O)C=CC(=C3)O)O</chem>	[22, 19]
18	Calycosin	5280448	<chem>COC1=C(C=C(C=C1)C2=COC3=C(C2=O)C=CC(=C3)O)O</chem>	[22]
19	Pyromucic acid	6919	<chem>C1=COC(=C1)C(=O)O</chem>	[22]
20	1,3,5-benzenetriol	359	<chem>C1=C(C=C(C=C1O)O)O</chem>	[22]
21	Succinic acid	1110	<chem>C(CC(=O)O)C(=O)O</chem>	[22]
22	Beta-sitosterol	222284	<chem>CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C(C)C</chem>	[22]
23	Stigmaterol	5280794	<chem>CCC(C=CC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C(C)C</chem>	[19]
24	(6aR,11aR)-medicarpin	336327	<chem>COC1=CC2=C(C=C1)C3COC4=C(C3O2)C=CC(=C4)O</chem>	
25	8-O-methylretusin	5319771	<chem>COC1=CC=C(C=C1)C2=COC3=C(C2=O)C=CC(=C3OC)O</chem>	[19]
26	Biochanin A	5280373	<chem>COC1=CC=C(C=C1)C2=COC3=CC(=CC(=C3C2=O)O)O</chem>	[19]

Continuation of Table 1

27	Trans-4-Hydroxymellein	10262028	CC1C(C2=C(C(=CC=C2)O)C(=O)O1)O	[19]
28	Cis-4-Hydroxymellein	10420140	CC1C(C2=C(C(=CC=C2)O)C(=O)O1)O	[19]
29	Coumestrol	5281707	C1=CC2=C(C=C1O)OC3=C2C(=O)OC4=C3C=CC(=C4)O	[19, 21]
30	Isoliquiritigenin	638278	C1=CC(=CC=C1C=CC(=O)C2=C(C=C(C=C2)O)O)O	[21]
31	Lupinalbin A	5324349	C1=CC2=C(C=C1O)OC3=C2C(=O)C4=C(C=C(C=C4O3)O)O	[21]
32	Leonuriside A	14237625	COC1=CC(=CC(=C1OC2C(C(C(C(O2)CO)O)O)O)OC)O	[21]
33	Suberectin	5321538	COC1=C(O)C=C2OC(CC(=O)C2=C1)C1=CC(O)=C(O)C=C1	[22]

Table 2 Apoptotic-related activity screening results

No	Compounds	Pa > 0.75					
		Caspase-3 stimulant	Antineoplastic	Apoptotic agonist	TP53 expression enhancer	Caspase-8 stimulant	Anti-carcinogenic
1	Maackiain	0.934	0.788	0.751	-	-	-
2	Medicarpin	0.991	0.769	0.778	0.774	-	-
3	Trigraecum	0.751	-	0.769	0.863	-	-
4	Sativan	-	-	-	-	-	-
5	Pseudobaptigenin	-	-	-	-	-	-
6	Genistin	0.763	0.814	0.757	0.912	-	0.951
7	Prestegane B	-	-	-	-	-	-
8	(+)-medioresinol	0.757	0.812	-	0.800	-	-
9	Benzeneethanol	-	-	-	-	-	-
10	4,7,2'-trihydroxy-4'-methoxyisoflavone	-	-	-	-	-	-
11	Naringenin	-	-	-	0.822	-	-
12	Blumenol A	-	-	-	-	-	-
13	Protocatechuic acid ethyl ester	-	-	-	-	-	-
14	Liquiritigenin	-	-	-	0.769	-	-
15	protocatechuic acid	-	-	-	-	-	-
16	Glycyroside	0.975	0.864	0.790	-	0.857	0.922
17	Formononetin	-	-	-	0.770	-	-
18	Daidzein	-	-	0.755	0.771	-	-
19	Calycosin	-	-	0.779	0.797	-	-
20	Pyromucic acid	-	-	-	-	-	-
21	1,3,5-benzenetriol	-	-	-	0.752	-	-
22	Succinic acid	-	-	-	-	-	-
23	Beta-sitosterol	0.806	-	-	-	-	-
24	Stigmasterol	0.863	-	0.753	-	-	-
25	8-O-methylretusin	-	-	0.827	-	-	-
26	Biochanin A	-	-	0.823	0.823	-	-
27	Trans-4-Hydroxymellein	-	0.790	-	0.796	-	-
28	Cis-4-Hydroxymellein	-	0.790	-	0.796	-	-
29	Coumestrol	-	-	0.811	0.769	-	-
30	Isoliquiritigenin	0.793	0.752	0.812	-	-	-
31	lupinalbin A	-	0.953	-	0.789	-	-
32	Leonuriside A	0.862	0.829	0.712	0.835	0.762	0.833
33	Suberectin	0.751	-	-	0.780	-	-

Table 3 Four highest Pa value (>0.75) related to apoptotic

Biological activity	Four highest Pa value
Caspase-3 stimulant	Medicarpin (0.991), glycyroside (0.975), maackiain (0.934), Stigmasterol (0.863)
Antineoplastic	lupinalbin A (0.953), glycyroside (0.864), leonuriside A (0.829), medioresinol (0.812)
Apoptotic agonist	8-O-methylretusin (0.827), biochanin A (0.823), isoliquiritigenin (0.812), coumestrol (0.811)
TP53 expression enhancer	genistin (0.911), trigraecum (0.863), leonuriside A (0.835), biochanin A (0.823)
Caspase 8 stimulant	glycyroside (0.857), leonuriside A (0.762)
Anticarcinogenic	genistin (0.951), glycyroside (0.922), Leonuriside A(0.833)

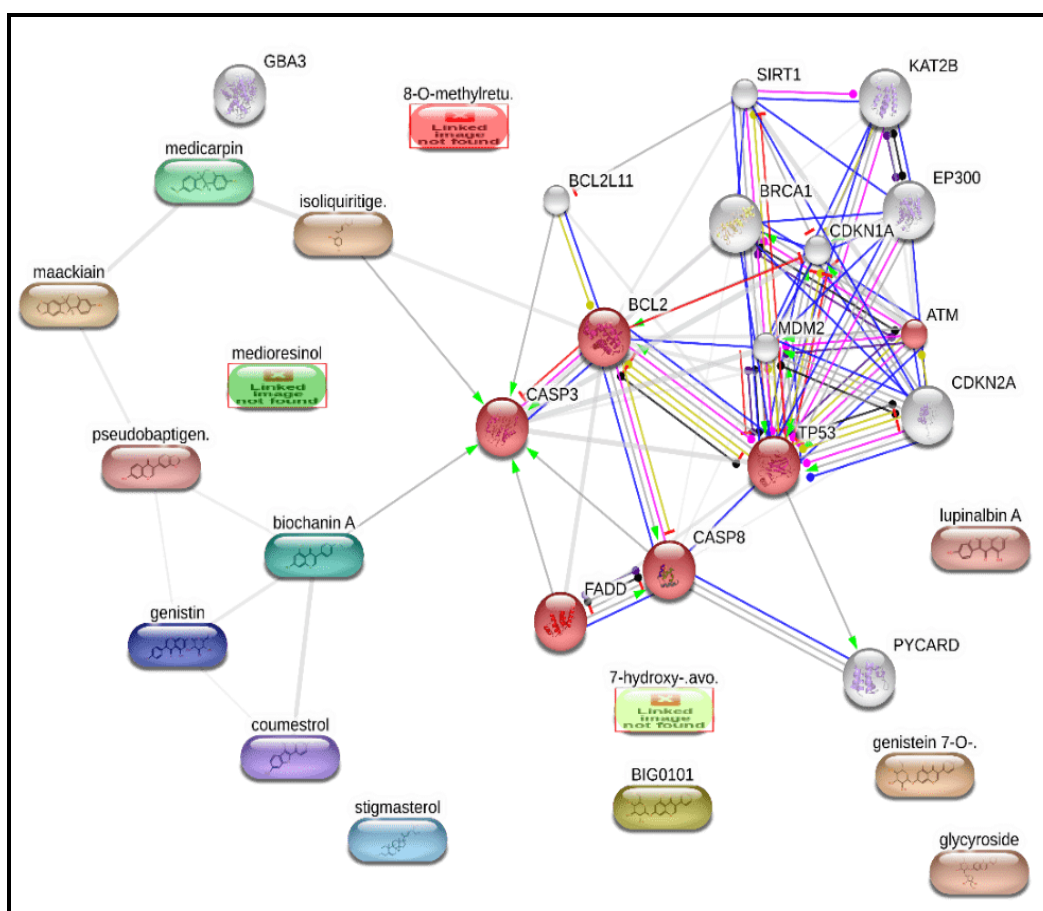


Fig. 1 Apoptotic pathway

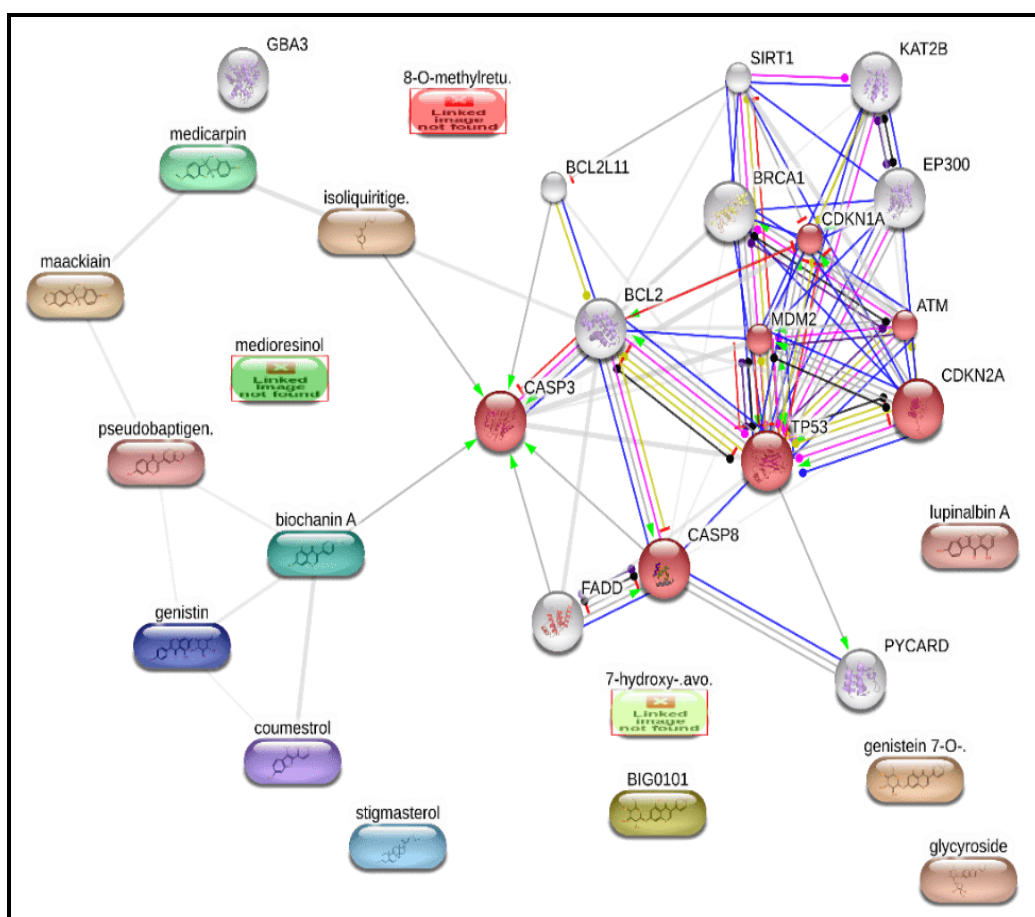


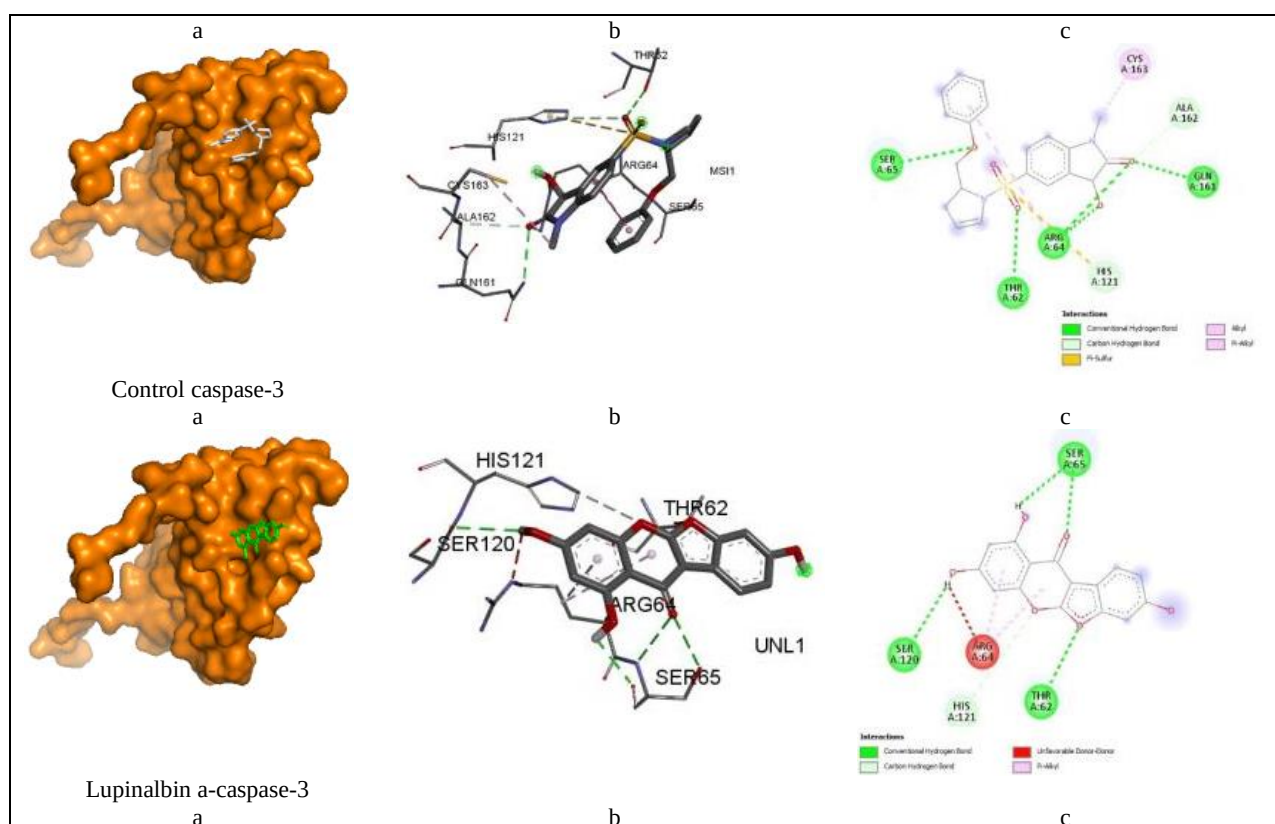
Fig. 2 p53 signaling pathway

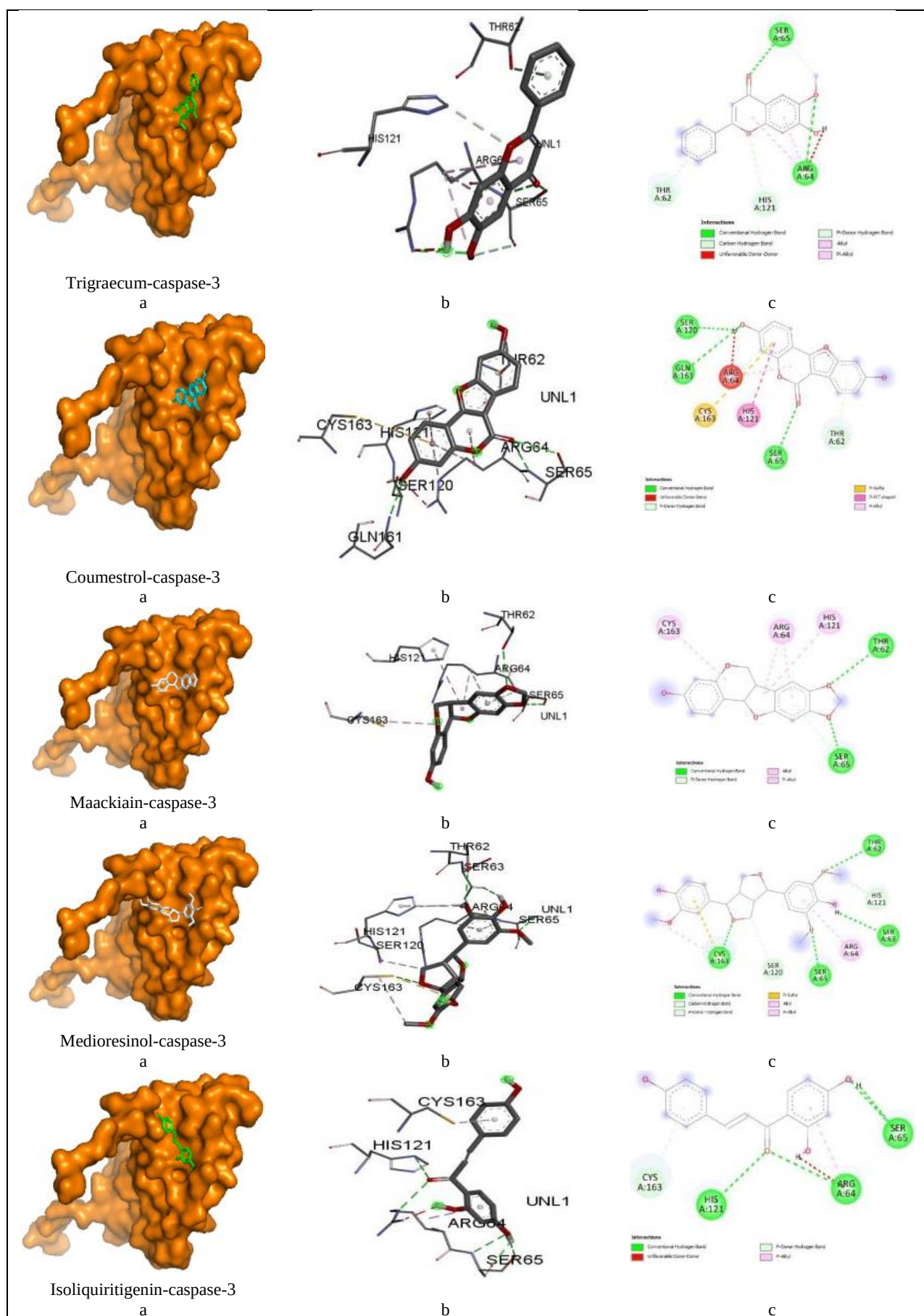
Table 4 Results of the analysis of pharmacological properties

No	Compound	Molecular weight (g/mol)	LogP values	Solubility (Log S)	Flexibility			Lipophilicity (Log P)
					H-bond acceptors	H-bond donors	Rotatable bonds	
1	Medicarpin	270.28	47.92 Å ²	-3.64	4	1	1	2.77
2	Glycyroside	562.52	197.74 Å ²	-2.95	13	6	8	-0.23
3	Maackiain	284.26	57.15 Å ²	-3.67	5	1	0	2.61
4	Stigmasterol	412.69	20.23 Å ²	-7.46	1	1	5	8.56
5	Lupinalbin A	284.22	104.04 Å ²	-4.2	6	3	0	3.18
6	Leonuriside A	332.3	138.07 Å ²	-1.2	9	5	5	-0.90
7	Medioresinol	388.41	86.61 Å ²	-3.65	7	2	5	2.25
8	8-O-methylretusin	298.29	68.90 Å ²	-3.77	5	1	3	2.77
9	Biochanin A	284.26	79.90 Å ²	-3.92	5	2	2	2.99
10	Isoliquiritigenin	256.25	77.76 Å ²	-3.7	4	3	3	3.18
11	Coumestrol	268.22	83.81 Å ²	-3.87	5	2	0	2.76
12	Genistin	432.38	170.05 Å ²	-3.18	10	6	4	0.86
13	Trigraecum	268.26	59.67 Å ²	-4.22	4	1	2	3.59

Table 5 Binding affinity of nine chosen compounds of genus Spatholobus

Compounds	Binding affinity (kcal/mol)	
	Caspase-3	
1-methyl-5-(2-phenoxyethyl-pyrrolidine-1-sulfonyl)-1h-indole-2,3-dione (MSI/control)	-5.4	
Lupinalbin A	-5.5	
Trigraecum	-5.5	
Coumestrol	-5.3	
Maackiain	-5.2	
Medioresinol	-5.2	
Isoliquiritigenin	-5.0	
8-O-methylretusin	-4.9	
Biochanin A	-4.9	
Medicarpin	-4.7	





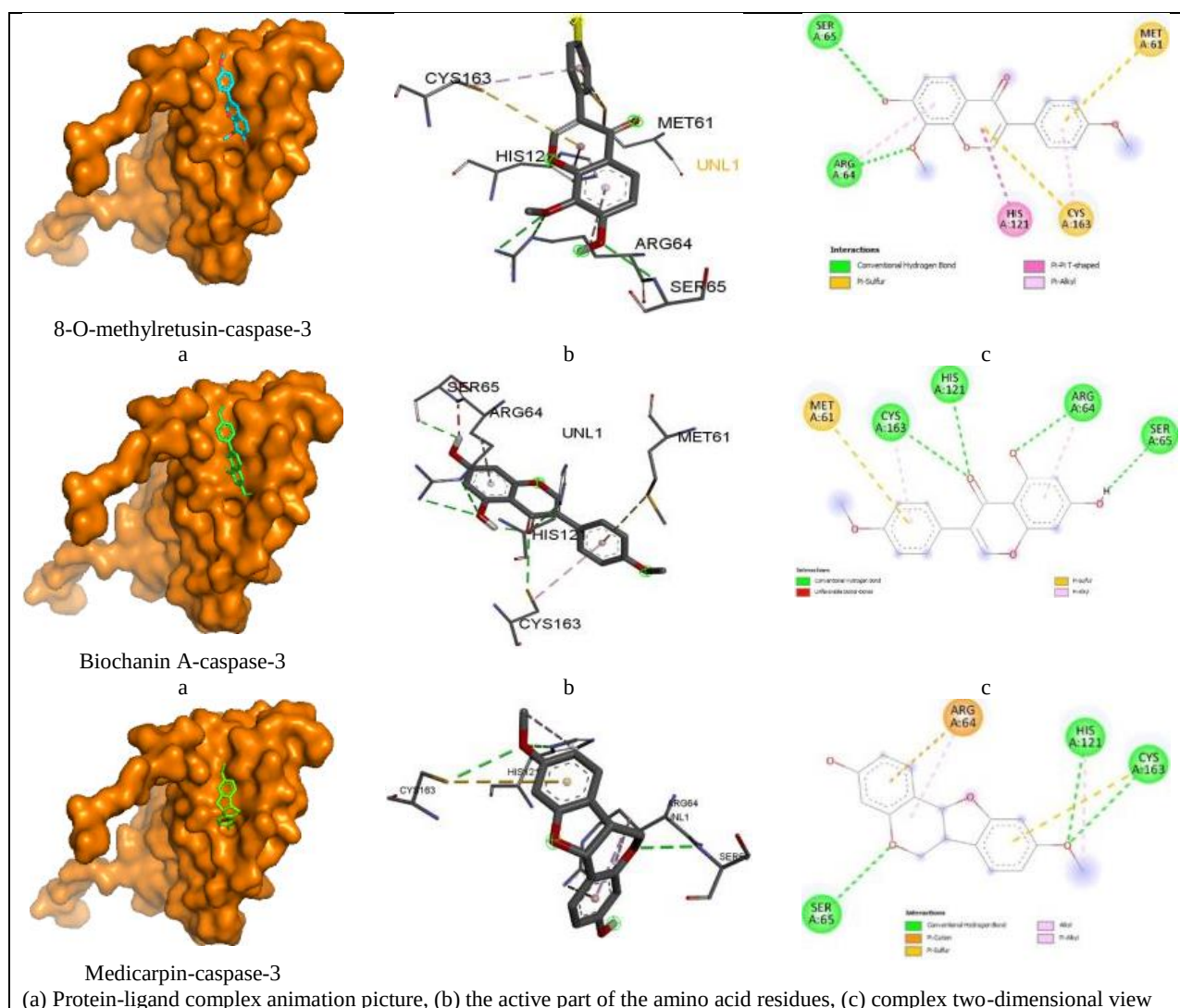


Fig. 3 Ligand-protein complexes

Name	Visible	Color	Parent	Distance	Category	Types
1 A:THR62:OG1 - A:MSI1:O4	✓ Yes	Green	Ligand Non-bond Monitor	2.87578	Hydrogen Bond	Conventional Hydrogen Bond
2 A:ARG64:NE - A:MSI1:O3	✓ Yes	Green	Ligand Non-bond Monitor	2.95204	Hydrogen Bond	Conventional Hydrogen Bond
3 A:ARG64:NH2 - A:MSI1:O2	✓ Yes	Green	Ligand Non-bond Monitor	3.1977	Hydrogen Bond	Conventional Hydrogen Bond
4 A:SER65:N - A:MSI1:O1	✓ Yes	Green	Ligand Non-bond Monitor	3.02727	Hydrogen Bond	Conventional Hydrogen Bond
5 A:GLN161:NE2 - A:MSI1:O2	✓ Yes	Green	Ligand Non-bond Monitor	3.02539	Hydrogen Bond	Conventional Hydrogen Bond
6 A:HIS121:CE1 - A:MSI1:O4	✓ Yes	Green	Ligand Non-bond Monitor	3.44982	Hydrogen Bond	Carbon Hydrogen Bond
7 A:ALA162:CA - A:MSI1:O2	✓ Yes	Green	Ligand Non-bond Monitor	3.60093	Hydrogen Bond	Carbon Hydrogen Bond
8 A:MSI1:S1 - A:HIS121	✓ Yes	Yellow	Ligand Non-bond Monitor	5.58769	Other	Pi-Sulfur
9 A:MSI1 - A:MSI1	✓ Yes	Pink	Ligand Non-bond Monitor	3.87583	Hydrophobic	Pi-Pi Stacked
10 A:MSI1:C1 - A:CYS163	✓ Yes	Pink	Ligand Non-bond Monitor	4.91414	Hydrophobic	Alkyl
11 A:MSI1 - A:ARG64	✓ Yes	Pink	Ligand Non-bond Monitor	5.4687	Hydrophobic	Pi-Alkyl

Fig. 4 MSI-caspase-3 interactions

Name	Visible	Color	Parent	Distance	Category	Types
1 A:THR62:OG1 - :UNL1:O	✓ Yes	Green	Ligand Non-bond Monitor	2.84391	Hydrogen Bond	Conventional Hydrogen Bond
2 A:SER65:N - :UNL1:O	✓ Yes	Green	Ligand Non-bond Monitor	3.14708	Hydrogen Bond	Conventional Hydrogen Bond
3 A:SER65:OG - :UNL1:O	✓ Yes	Green	Ligand Non-bond Monitor	2.84356	Hydrogen Bond	Conventional Hydrogen Bond
4 :UNL1:H - A:SER65:O	✓ Yes	Green	Ligand Non-bond Monitor	2.40886	Hydrogen Bond	Conventional Hydrogen Bond
5 :UNL1:H - A:SER120:O	✓ Yes	Green	Ligand Non-bond Monitor	2.89309	Hydrogen Bond	Conventional Hydrogen Bond
6 A:HIS121:CE1 - :UNL1:O	✓ Yes	Green	Ligand Non-bond Monitor	3.63639	Hydrogen Bond	Carbon Hydrogen Bond
7 :UNL1 - A:ARG64	✓ Yes	Pink	Ligand Non-bond Monitor	4.5798	Hydrophobic	Pi-Alkyl
8 :UNL1 - A:ARG64	✓ Yes	Pink	Ligand Non-bond Monitor	3.80441	Hydrophobic	Pi-Alkyl

Fig. 5 Lupinalbin A-caspase-3 interactions

	Name	Visible	Color	Parent	Distance	Category	Types
1	A:ARG64:NH2 - :UNL1:O	☒ Yes		Ligand Non-bond Monitor	2.98432	Hydrogen Bond	Conventional Hydrogen Bond
2	A:SER65:N - :UNL1:O	☒ Yes		Ligand Non-bond Monitor	3.07322	Hydrogen Bond	Conventional Hydrogen Bond
3	A:SER65:OG - :UNL1:O	☒ Yes		Ligand Non-bond Monitor	2.83035	Hydrogen Bond	Conventional Hydrogen Bond
4	A:HIS121:CE1 - :UNL1:O	☒ Yes		Ligand Non-bond Monitor	3.65641	Hydrogen Bond	Carbon Hydrogen Bond
5	:UNL1:C - A:SER65:O	☒ Yes		Ligand Non-bond Monitor	3.28245	Hydrogen Bond	Carbon Hydrogen Bond
6	A:THR62:OG1 - :UNL1	☒ Yes		Ligand Non-bond Monitor	3.40677	Hydrogen Bond	Pi-Donor Hydrogen Bond
7	:UNL1:C - A:ARG64	☒ Yes		Ligand Non-bond Monitor	4.2964	Hydrophobic	Alkyl
8	:UNL1 - A:ARG64	☒ Yes		Ligand Non-bond Monitor	4.71568	Hydrophobic	Pi-Alkyl
9	:UNL1 - A:ARG64	☒ Yes		Ligand Non-bond Monitor	3.88577	Hydrophobic	Pi-Alkyl

Fig. 6 Trigraecum-caspase-3 interactions

	Name	Visible	Color	Parent	Distance	Category	Types
1	A:SER65:...	☒ Yes		Ligand Non-bond Monitor	2.95405	Hydrogen Bond	Conventional Hydrogen Bond
2	A:SER65:...	☒ Yes		Ligand Non-bond Monitor	3.03504	Hydrogen Bond	Conventional Hydrogen Bond
3	A:GLN16...	☒ Yes		Ligand Non-bond Monitor	3.22306	Hydrogen Bond	Conventional Hydrogen Bond
4	:UNL1:H - ...	☒ Yes		Ligand Non-bond Monitor	2.40531	Hydrogen Bond	Conventional Hydrogen Bond
5	A:THR62:...	☒ Yes		Ligand Non-bond Monitor	3.32003	Hydrogen Bond	Pi-Donor Hydrogen Bond
6	A:ARG64...	☒ Yes		Ligand Non-bond Monitor	3.99645	Hydrogen Bond	Pi-Donor Hydrogen Bond
7	A:CYS16...	☒ Yes		Ligand Non-bond Monitor	5.13825	Other	Pi-Sulfur
8	A:HIS121...	☒ Yes		Ligand Non-bond Monitor	4.86047	Hydrophobic	Pi-Pi T-shaped
9	:UNL1 - A...	☒ Yes		Ligand Non-bond Monitor	4.2775	Hydrophobic	Pi-Alkyl
10	:UNL1 - A...	☒ Yes		Ligand Non-bond Monitor	4.6308	Hydrophobic	Pi-Alkyl

Fig. 7 Coumestrol-caspase-3 interactions

	Name	Visible	Color	Parent	Distance	Category	Types
1	A:THR62:OG1 - :UNL1:O	☒ Yes		Ligand Non-bond Monitor	3.02672	Hydrogen Bond	Conventional Hydrogen Bond
2	A:SER65:OG - :UNL1:O	☒ Yes		Ligand Non-bond Monitor	2.99719	Hydrogen Bond	Conventional Hydrogen Bond
3	A:SER65:N - :UNL1	☒ Yes		Ligand Non-bond Monitor	4.15684	Hydrogen Bond	Pi-Donor Hydrogen Bond
4	A:ARG64 - :UNL1	☒ Yes		Ligand Non-bond Monitor	3.84283	Hydrophobic	Alkyl
5	A:CYS163 - :UNL1	☒ Yes		Ligand Non-bond Monitor	5.35575	Hydrophobic	Alkyl
6	A:HIS121 - :UNL1	☒ Yes		Ligand Non-bond Monitor	5.28664	Hydrophobic	Pi-Alkyl
7	:UNL1 - A:ARG64	☒ Yes		Ligand Non-bond Monitor	4.19047	Hydrophobic	Pi-Alkyl

Fig. 8 Maackia-caspase-3 interactions

	Name	Visible	Color	Parent	Distance	Category	Types
1	A:THR62:OG1 - :UNL1:O	☒ Yes		Ligand Non-bond Monitor	2.83267	Hydrogen Bond	Conventional Hydrogen Bond
2	A:SER65:OG - :UNL1:O	☒ Yes		Ligand Non-bond Monitor	2.70235	Hydrogen Bond	Conventional Hydrogen Bond
3	A:CYS163:SG - :UNL1:O	☒ Yes		Ligand Non-bond Monitor	3.64806	Hydrogen Bond	Conventional Hydrogen Bond
4	:UNL1:H - A:SER63:O	☒ Yes		Ligand Non-bond Monitor	2.46442	Hydrogen Bond	Conventional Hydrogen Bond
5	A:HIS121:CE1 - :UNL1:O	☒ Yes		Ligand Non-bond Monitor	3.41152	Hydrogen Bond	Carbon Hydrogen Bond
6	:UNL1:C - A:SER120:O	☒ Yes		Ligand Non-bond Monitor	3.74833	Hydrogen Bond	Carbon Hydrogen Bond
7	A:SER65:N - :UNL1	☒ Yes		Ligand Non-bond Monitor	4.1737	Hydrogen Bond	Pi-Donor Hydrogen Bond
8	A:CYS163:SG - :UNL1	☒ Yes		Ligand Non-bond Monitor	3.90601	Other	Pi-Sulfur
9	:UNL1:C - A:CYS163	☒ Yes		Ligand Non-bond Monitor	4.40746	Hydrophobic	Alkyl
10	A:HIS121 - :UNL1:C	☒ Yes		Ligand Non-bond Monitor	4.86624	Hydrophobic	Pi-Alkyl
11	:UNL1 - A:ARG64	☒ Yes		Ligand Non-bond Monitor	4.03388	Hydrophobic	Pi-Alkyl

Fig. 9 Medioresinol-caspase-3 interactions

	Name	Visible	Color	Parent	Distance	Category	Types
1	A:ARG64:NE - :UNL1:O	☒ Yes		Ligand Non-bond Monitor	2.93039	Hydrogen Bond	Conventional Hydrogen Bond
2	A:SER65:N - :UNL1:O	☒ Yes		Ligand Non-bond Monitor	2.90309	Hydrogen Bond	Conventional Hydrogen Bond
3	A:SER65:OG - :UNL1:O	☒ Yes		Ligand Non-bond Monitor	2.93161	Hydrogen Bond	Conventional Hydrogen Bond
4	A:HIS121:ND1 - :UNL1:O	☒ Yes		Ligand Non-bond Monitor	3.14281	Hydrogen Bond	Conventional Hydrogen Bond
5	:UNL1:H - A:SER65:O	☒ Yes		Ligand Non-bond Monitor	2.21606	Hydrogen Bond	Conventional Hydrogen Bond
6	A:CYS163:SG - :UNL1	☒ Yes		Ligand Non-bond Monitor	3.79127	Hydrogen Bond;Other	Pi-Donor Hydrogen Bond;Pi-Sulfur
7	:UNL1 - A:ARG64	☒ Yes		Ligand Non-bond Monitor	3.95478	Hydrophobic	Pi-Alkyl

Fig. 10 Isoliquiritigenin-caspase-3 interactions

	Name	Visible	Color	Parent	Distance	Category	Types
1	A:ARG64...	☒ Yes		Ligand Non-bond Monitor	3.29203	Hydrogen Bond	Conventional Hydrogen Bond
2	A:ARG64...	☒ Yes		Ligand Non-bond Monitor	3.13906	Hydrogen Bond	Conventional Hydrogen Bond
3	A:SER65:...	☒ Yes		Ligand Non-bond Monitor	3.16316	Hydrogen Bond	Conventional Hydrogen Bond
4	A:MET61:...	☒ Yes		Ligand Non-bond Monitor	5.22337	Other	Pi-Sulfur
5	A:CYS16...	☒ Yes		Ligand Non-bond Monitor	5.00118	Other	Pi-Sulfur
6	A:HIS121...	☒ Yes		Ligand Non-bond Monitor	4.51993	Hydrophobic	Pi-Pi T-shaped
7	:UNL1 - A...	☒ Yes		Ligand Non-bond Monitor	4.27625	Hydrophobic	Pi-Alkyl
8	:UNL1 - A...	☒ Yes		Ligand Non-bond Monitor	4.99271	Hydrophobic	Pi-Alkyl

Fig. 11 8-O-methylretusin-caspase-3 interactions

	Name	Visible	Color	Parent	Distance	Category	Types
1	A:ARG64:NE - :UNL1:O	☒ Yes		Ligand Non-bond Monitor	3.11202	Hydrogen Bond	Conventional Hydrogen Bond
2	A:ARG64:NH2 - :UNL1:O	☒ Yes		Ligand Non-bond Monitor	3.38887	Hydrogen Bond	Conventional Hydrogen Bond
3	A:HIS121:ND1 - :UNL1:O	☒ Yes		Ligand Non-bond Monitor	3.12312	Hydrogen Bond	Conventional Hydrogen Bond
4	A:CYS163:SG - :UNL1:O	☒ Yes		Ligand Non-bond Monitor	3.51055	Hydrogen Bond	Conventional Hydrogen Bond
5	:UNL1:H - :UNL1:O	☒ Yes		Ligand Non-bond Monitor	1.7913	Hydrogen Bond	Conventional Hydrogen Bond
6	:UNL1:H - A:SER65:O	☒ Yes		Ligand Non-bond Monitor	2.42998	Hydrogen Bond	Conventional Hydrogen Bond
7	A:MET61:SD - :UNL1	☒ Yes		Ligand Non-bond Monitor	5.08124	Other	Pi-Sulfur
8	:UNL1 - A:ARG64	☒ Yes		Ligand Non-bond Monitor	4.14095	Hydrophobic	Pi-Alkyl
9	:UNL1 - A:CYS163	☒ Yes		Ligand Non-bond Monitor	5.01979	Hydrophobic	Pi-Alkyl

Fig. 12 Biochanin A-caspase-3 interactions

	Name	Visible	Color	Parent	Distance	Category	Types
1	A:SER65:N - :UNL1:O	☒ Yes		Ligand Non-bond Monitor	3.28962	Hydrogen Bond	Conventional Hydrogen Bond
2	A:HIS121:ND1 - :UNL1:O	☒ Yes		Ligand Non-bond Monitor	3.35119	Hydrogen Bond	Conventional Hydrogen Bond
3	A:CYS163:SG - :UNL1:O	☒ Yes		Ligand Non-bond Monitor	3.69895	Hydrogen Bond	Conventional Hydrogen Bond
4	A:ARG64:NH2 - :UNL1	☒ Yes		Ligand Non-bond Monitor	3.79389	Electrostatic	Pi-Cation
5	A:CYS163:SG - :UNL1	☒ Yes		Ligand Non-bond Monitor	5.34761	Other	Pi-Sulfur
6	A:ARG64 - :UNL1	☒ Yes		Ligand Non-bond Monitor	4.92458	Hydrophobic	Alkyl
7	A:HIS121 - :UNL1:C	☒ Yes		Ligand Non-bond Monitor	4.67437	Hydrophobic	Pi-Alkyl
8	:UNL1 - A:ARG64	☒ Yes		Ligand Non-bond Monitor	5.27431	Hydrophobic	Pi-Alkyl

Fig. 13 Medicarpin-caspase-3 interactions

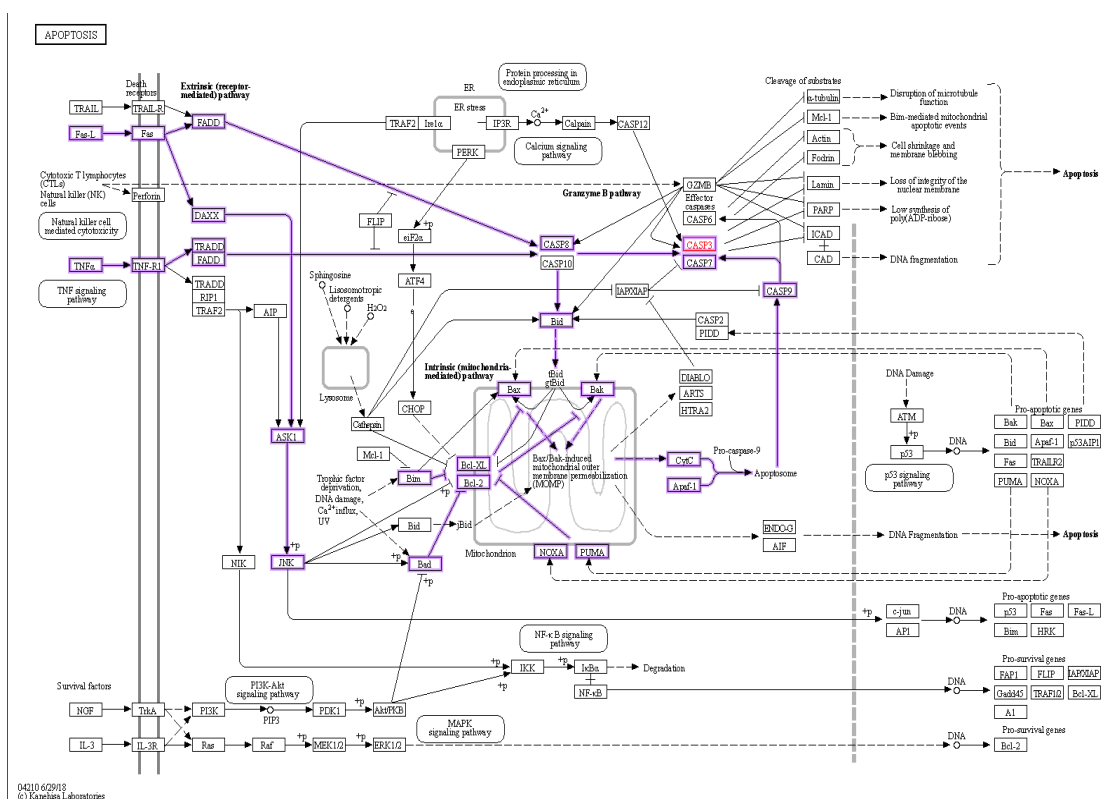


Fig. 14 Apoptotic pathway of caspase-3 [24]

In addition, according to the apoptotic pathway, isoliquiritinigen interacts with Bcl2 directly (70.0%), but there is no explanation about the interaction type. Meanwhile, the other ligands show no interaction with proteins on both pathways. We assume that the database of STITCH is not updated yet, or the latest report is unavailable.

3.4. SwissADME Analysis

The list of pharmacological properties of active compounds is presented in Table 4. Based on the optimum criteria, glycyroside (MW>500 g, TPSA>130 Å²), stigmasterol (Log S< -6, Log P3>5), leonuride A (TPSA>130 Å²), and genistin (TPSA>130 Å²) do not meet the optimum criteria of pharmacological properties. In contrast, the others are under the appropriate criteria.

3.5. Molecular Docking Analysis

The caspase-3 protein (PDB ID 1GFU) has active sites on HIS 121 and Cyst (cysteine) 163 in A Chain. The active site of this protein is indicated by the type of interaction between protein and ligand acetamide derivative. These interactions occur in the A chain; the types of interaction are Pi-sulfur cysteine 163, Pi-Pi T-shaped HIS 121, and pi-cation HIS 121 [23]. Docking results also show that natural ligands or compounds interact with the active site of amino acid residues.

Control ligand (MSI) interacts with HIS 121 (carbon-hydrogen bond and pi-sulfur) and Cys 163 (alkyl). Lupinalbin A interacts with HIS 121 (carbon-hydrogen bond), trigraecum – with HIS121 (carbon-hydrogen bond), coumestrol – with Cys163 (pi-sulfur)

and HIS 121 (Pi-Pi T-shaped), maackiain – with Cys 163 (alkyl), and HIS 121 (Pi-Alkyl). Medioresinol interacts with Cys 163 and HIS 121 in conventional hydrogen, carbon-hydrogen, and pi alkyl bonds. Isoliquiritigenin links HIS 121 and Cys 163 through conventional hydrogen, pi-donor hydrogen, and pi-sulfur bonds. At the same time, 8-o-methylretusin interacts with Cys 163 (Pi-Sulfur) and HIS 121 (Pi-Pi T-shaped). Biochanin interacts with HIS 121 (Conventional Hydrogen Bond) and Cys 163 (Conventional Hydrogen Bond), medicarpin – with HIS 121 (Conventional Hydrogen Bond), Cys 163 (Conventional Hydrogen Bond), and HIS 121 (Pi-Alkyl) (Figs. 4-13).

Overall, all ligands interact with the active site of the protein caspase-3. However, other amino acid residues apart from HIS 121 and Cys 136 interact with natural ligands. The molecular docking analysis shows that the highest binding affinity against caspase-3 is found in the lupinalbin A and trigacum (-5.5 kcal/mol). Meanwhile, other ligands have binding affinity under their control ligands (Table 5). Thus, both lupinalbin A and trigacum compounds have the highest potential as caspase-3 activator agents and suppress breast cancer cell growth.

Caspase-3 protein (PDB ID 1GFU) was also used as a target model in molecular docking studies with compounds from *Moringa oleifera* fruit. The results show that these compounds have anticancer activity, and there is a match between the molecular docking simulation test and the in vitro test [24].

4. Conclusion

Based on the analysis results of various procedures, caspase-3 is probably as target protein in the apoptotic and p53 signaling pathways. Due to software limitations, the presence of water molecules in the molecular docking was intentionally ignored. So in further study, this needs to be validated with dynamic molecular simulations. In addition, in vitro study of apoptotic cancer cell models is also required to support the provisional hypothesis that caspase-3 can be activated by bioactive compounds from plants of the genus *Spatholobus*. Furthermore, the estimated nine compounds that meet the optimum criteria to be consumed orally are medicarpin, maackiain, lupinalbin A, medioresinol, 8-O-methylretusin, biochanin A, isoliquiritigenin, coumestrol, and trigraecum. These compounds are probably nominated as protein activators of caspase-3 in both pathways.

References

- [1] WHO. THE GLOBAL CANCER OBSERVATORY. *Cancer Incident in Indonesia*. International Agency for Research on Cancer, 2020. [Online]. Available from: <https://gco.iarc.fr/>.
- [2] PISTRITTO G., TRISCIUOGLIO D., CECI C., GARUFI A., and D'ORAZI G. Apoptosis as anticancer mechanism: function and dysfunction of its modulators and targeted therapeutic strategies. *Aging*, 2016, 8(4): 603-619, DOI: 10.18632/aging.100934.
- [3] ARAYA L.E., SONI I.V., HARDY J.A., and JULIEN O. Deorphanizing Caspase-3 and Caspase-9 Substrates In and Out of Apoptosis with Deep Substrate Profiling. *ACS Chemical Biology*, 2021, 16(11): 2280-2296. DOI: 10.1021/acscchembio.1c00456.
- [4] AN H., HEO J.S., OOSHIMA A., WU Z., KIM S.-J., BAE I., and YANG K.-M. Abstract 989: Tetraarsenic hexoxide enhances generation of mitochondrial ROS to promote pyroptosis by inducing the activation of caspase-3/GSDME in triple-negative breast cancer cells. In: *Proceedings: AACR Annual Meeting 2021; April 10-15, 2021 and May 17-21, 2021; Philadelphia, PA*, 2021. DOI: 10.1158/1538-7445.AM2021-989.
- [5] ESKANDARI E., EAVES C.J., and TAN S. Abstract 1949: Caspase-3 plays a requisite role in regulating survival of human breast cancer cells. In: *Proceedings: AACR Annual Meeting 2021; April 10-15, 2021 and May 17-21, 2021; Philadelphia, PA*, 2021. DOI: 10.1158/1538-7445.AM2021-1949.
- [6] CHEN L., ZENG Y., and ZHOU S.-F. Role of Apoptosis in Cancer Resistance to Chemotherapy. In: TUTAR Y. (ed.) *Current Understanding of Apoptosis*, 2018. DOI: 10.5772/intechopen.80056.
- [7] THAPA S., RATHER R.A., SINGH S.K., and BHAGAT M. Insights into the Role of Defective Apoptosis in Cancer Pathogenesis and Therapy. In: TUTAR Y. (ed.) *Regulation and Dysfunction of Apoptosis [Working Title]*, IntechOpen, 2021. DOI: 10.5772/intechopen.97536
- [8] CHEN F., ZHONG Z., TAN H.Y., WANG N., and FENG Y. The Underlying Mechanisms of Chinese Herbal Medicine-Induced Apoptotic Cell Death in Human Cancer. In: GALI-MUHTASIB H., and RAHAL O.N. (eds.) *Programmed Cell Death*, 2020.
- [9] CRAGG G.M., and PEZZUTO J.M. Natural Products as a Vital Source for the Discovery of Cancer Chemotherapeutic and Chemopreventive Agents. *Medical Principles and Practice*, 2016, 25(2): 41-59. DOI: 10.1159/000443404.
- [10] YANAGIHARA K., ITO A., TOGE T., and NUMOTO M. Antiproliferative Effects of Isoflavones on Human Cancer Cell Lines Established from the Gastrointestinal Tract. *Cancer Research*, 1993, 53(23): 5815-5821.
- [11] EMAMI S., and GHANBARIMASIR Z. Recent advances of chroman-4-one derivatives: Synthetic approaches and bioactivities. *European Journal of Medicinal Chemistry*, 2015, 93: 539-563. DOI: 10.1016/j.ejmech.2015.02.048.
- [12] TAY K.-C., TAN L.T.-H., CHAN C.K., HONG S.L., CHAN K.-G., YAP W.H., PUSPARAJAH P., LEE L.-H., and GOH B.-H. Formononetin: A Review of Its Anticancer Potentials and Mechanisms. *Frontiers in Pharmacology*, 2019, 10: 820. DOI: 10.3389/fphar.2019.00820.
- [13] THE PLANT LIST. *Species in Spatholobus*. [Online]. Available from: <http://www.theplantlist.org/1.1/browse/A/Leguminosae/Spatholobus/>.
- [14] CHRISTINA Y.I., NAFISAH W., ATHO'ILLAH M.F., RIFA'I M., WIDODO N., and DJATI M.S. Anti-breast cancer potential activity of *Phaleria macrocarpa* (Scheff.) Boerl. leaf extract through in silico studies. *Journal of Pharmacy and Pharmacognosy Research*, 2021, 9(6): 824-845.
- [15] LAGUNIN A.A., DUBOVSKAJA V.I., RUDIK A.V., POGODIN P.V., DRUZHILOVSKIY D.S., GLORIOZOVA T.A., FILIMONOV D.A., SASTRY N.G., and POROIKOV V.V. CLC-Pred: A freely available web-service for in silico prediction of human cell line cytotoxicity for drug-like compounds. *PLOS ONE*, 2018, 13(1): e0191838. DOI: 10.1371/journal.pone.0191838.
- [16] DAINA A., MICHELIN O., and ZOETE V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, 2017, 7(10): 1-13. DOI: 10.1038/srep42717.
- [17] YOON J.S., SUNG S.H., PARK J.H., and KIM Y.C. Flavonoids from *Spatholobus suberectus*. *Archives of Pharmacal Research*, 2004, 27(6): 589-592. DOI: 10.1007/BF02980154.
- [18] YIN T., LIU H., WANG B., TU G.-Z., LIANG H., and ZHAO Y.-Y. Chemical constituents from *Spatholobus sinensis*. *Acta Pharmaceutica Sinica*, 2008, 43(1): 67-70. <http://www.ncbi.nlm.nih.gov/pubmed/18357735>.
- [19] SICHAEM J., RUKSILP T., SAWASDEE P., KHUMKRATOK S., and TIPPYANG S. Chemical Constituents of the Stems of *Spatholobus parviflorus* and Their Cholinesterase Inhibitory Activity. *Chemistry of Natural Compounds*, 2018, 54(2): 356-357. DOI: 10.1007/s10600-018-2344-9.
- [20] TANG R.-N., QU X.-B., GUAN S.-H., XU P.-P., SHI Y.-Y., and GUO D.-A. Chemical constituents of *Spatholobus suberectus*. *Chinese Journal of Natural Medicines*, 2012, 10(1): 32-35. DOI: 10.1016/S1875-5364(12)60007-7.
- [21] CUI Y., LIU P., and CHEN R. Studies on the chemical constituents of *Spatholobus suberectus* Dunn. *Acta Pharmaceutica Sinica*, 2002, 37(10): 784-787. <http://www.ncbi.nlm.nih.gov/pubmed/12567862>.

- [22] MUTAZAH R., HAMID H.A., RAMLI A.N.M., ALUWI F.M., and YUSOFF M.M. In vitro cytotoxicity of Clinacanthus nutans fractions on breast cancer cells and molecular docking study of sulfur-containing compounds against caspase-3, *Food and Chemical Toxicology*, 2020, 135: 110869. DOI: 10.1016/j.fct.2019.110869.
- [23] FIROOZPOUR L., GAO L., MOGHIMI S., PASALAR P., DAVOODI J., WANG M.-W., REZAEI Z., DADGAR A., YAHYAVI H., AMANLOU M., and FOROUMADI A. Efficient synthesis, biological evaluation, and docking study of isatin based derivatives as caspase inhibitors. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 2020, 35(1): 1674-1684. DOI: 10.1080/14756366.2020.1809388.
- [24] SIDDIQUI S., UPADHYAY S., AHMAD I., HUSSAIN A., and AHAMED M. Cytotoxicity of Moringa oleifera fruits on human liver cancer and molecular docking analysis of bioactive constituents against caspase-3 enzyme. *Journal of Food Biochemistry*, 2021, 45(5). DOI: 10.1111/jfbc.13720.

參考文：

- [1]世界衛生組織。全球癌症觀察站。印度尼西亞的癌症事件。國際癌症研究機構，2020。[在線]。可從以下網址獲得：<https://gco.iarc.fr/>。
- [2] PISTRITTO G., TRISCIUOGGIO D., CECI C., GARUFI A. 和 D'ORAZI G. 細胞凋亡作為抗癌機制：其調節劑的功能和功能障礙以及靶向治療策略。老化，2016，8(4): 603-619, DOI: 10.18632/aging.100934。
- [3] ARAYA L.E., SONI I.V., HARDY J.A. 和 JULIEN O. 通過深度底物分析使 半胱天冬酶 -3 和 半胱天冬酶 -9 底物在細胞凋亡中進出脫孤。美國化學會化學生物學，2021，16(11): 2280-2296. DOI : 10.1021/acscchembio.1c00456。
- [4] AN H., HEO J.S., OOSHIMA A., WU Z., KIM S.-J., BAE I., 和 YANG K.-M.摘要 989：六氧化二砷通過誘導三陰性乳腺癌細胞中半胱天冬酶-3/加斯德明E的活化，促進線粒體活性氧的產生，從而促進細胞焦亡。在：會議記錄：2021年美國癌症研究協會年會；2021年4月10-15日和2021年5月17-21日；賓夕法尼亞州費城，2021年。DOI : 10.1158/1538-7445.AM2021-989。
- [5] ESKANDARI E., EAVES C.J. 和 TAN S. 摘要 1949：半胱天冬酶3在調節人類乳腺癌細胞的存活中起著必要的作用。在：會議記錄：2021年美國癌症研究協會年會；2021年4月10-15日和2021年5月17-21日；賓夕法尼亞州費城，2021年。DOI : 10.1158/1538-7445.AM2021-1949。
- [6] CHEN L., ZENG Y., 和 ZHOU S.-F. 細胞凋亡在癌症對化療耐藥中的作用。在：TUTAR Y. (編。) 目前對細胞凋亡的理解，2018。DOI: 10.5772/intechopen.80056。
- [7] THAPA S., RATHER R.A., SINGH S.K. 和 BHAGAT M.洞察缺陷性細胞凋亡在癌症發病機制和治療中的作用。在：TUTAR Y. (編。) 細胞凋亡的調節和功能障礙[工作名稱]，2021年。DOI : 10.5772/intechopen.97536
- [8] CHEN F., ZHONG Z., TAN H.Y., WANG N., & FENG

- Y. 中草藥誘導人類癌症細胞凋亡的潛在機制。在：GALIMUHTASIB H., & RAHAL O.N. (編輯) 程序性細胞死亡，2020年。
- [9] CRAGG G.M. 和 PEZZUTO J.M. 天然產品作為發現癌症化療和化學預防劑的重要來源。醫學原理與實踐，2016，25 (2) : 41-59. DOI : 10.1159/000443404。
- [10] YANAGIHARA K., ITO A., TOGE T. 和 NUMOTO M.異黃酮對從胃腸道建立的人類癌細胞系的抗增殖作用。癌症研究，1993，53 (23) : 5815-5821。
- [11] EMAMI S., 和 GHANBRIMASIR Z. 色滿-4-一衍生物的最近進展：合成方法和生物活性。歐洲藥物化學雜誌，2015，93 : 539-563. DOI : 10.1016/j.ejmech.2015.02.048。
- [12] TAY K.-C., TAN L.T.-H., CHAN C.K., HONG S.L., CHAN K.-G., YAP W.H., PUSPARAJAH P., LEE L.-H., 和 GOH B.H. 芒柄花素：其抗癌潛力和機制的回顧。藥理學前沿，2019，10: 820. DOI: 10.3389/fphar.2019.00820。
- [13]植物清單。物種在雞血藤。[在線的]。可從以下網址獲得：<http://www.theplantlist.org/1.1/browse/A/Leguminosae/Spatholobus/>。
- [14] CHRISTINA Y.I., NAFISAH W., ATHO'ILLAH M.F., RIFA'I M., WIDODO N., 和 DJATI M.S. 大果頭香的抗乳腺癌潛在活性。藥提取物通過計算機研究。藥學與生藥學研究雜誌，2021，9(6): 824-845。
- [15] LAGUNIN A.A., DUBOVSKAJA V.I., RUDIK A.V., POGODIN P.V., DRUZHILOVSKIY D.S., GLORIOZOVA T.A., FILIMONOV D.A., SASTRY N.G. 和 POROIKOV V.V. 細胞系細胞毒性預測器：一個免費提供的網絡服務，用於計算機預測類藥物化合物的人類細胞系細胞毒性。公共科學圖書館一，2018，13(1) : e0191838. DOI : 10.1371/journal.pone.0191838。
- [16] DAINA A., MICHIELIN O. 和 ZOETE V. 瑞士人ADME：一個免費的網絡工具，用於評估小分子的藥代動力學、藥物相似性和藥物化學友好性。科學報告，2017，7 (10) : 1-13. DOI : 10.1038/srep42717。
- [17] YOON J.S., SUNG S.H., PARK J.H. 和 KIM Y.C. 來自近直立的雞血藤的類黃酮。藥物研究檔案，2004，27(6): 589-592. DOI : 10.1007/BF02980154。
- [18] YIN T., LIU H., WANG B., TU G.-Z., LIANG H., 和 ZHAO Y.-Y. 雞血藤的化學成分。醫藥學報，2008，43(1): 67-70. <http://www.ncbi.nlm.nih.gov/pubmed/18357735>。
- [19] SICHAEM J., RUKSILP T., SAWASDEE P., KHUMKRATOK S. 和 TIPPYANG S. 小花小花莖的化學成分及其膽鹼酯酶抑制活性。天然化合物化學，2018，54(2): 356-357. DOI : 10.1007/s10600-018-2344-9。
- [20] TANG R.-N., QU X.-B., GUAN S.-H., XU P.-P., SHI Y.-Y., & GUO D.-A. 近直立的雞血藤的化學成分。中國天然藥物學報，2012，10(1): 32-35. DOI : 10.1016/S1875-5364(12)60007-7。
- [21] CUI Y., LIU P., & CHEN R. 化學成分研究近直立的雞血藤鄧恩。醫藥學報，2002，37(10): 784-787。

<http://www.ncbi.nlm.nih.gov/pubmed/12567862>。

[22] MUTAZAH R.、HAMID H.A.、RAMLI A.N.M.、ALUWI F.M. 和 YUSOFF M.M. 蒺藜組分對乳腺癌細胞的體外細胞毒性及含硫化合物對半胱天冬酶 -3 的分子對接研究, 食品與化學毒理學, 2020, 135: 110869. DOI: 10.1016/j.fct.2019.110869。

[23] FIROOZPOUR L., GAO L., MOGHIMI S., PASALAR P., DAVOODI J., WANG M.-W., REZAEI Z., DADGAR A., YAHYAVI H., AMANLOU M., 和 FOROUMADI A.

基於靛紅的衍生物作為半胱天冬酶抑制劑的高效合成、生物學評價和對接研究。酶抑制與藥物化學雜誌, 2020, 35(1): 1674-1684.

DOI : 10.1080/14756366.2020.1809388。

[24] SIDDIQUI S., UPADHYAY S., AHMAD I., HUSSAIN A., 和 AHAMED M. 辣木果實對人類肝癌的細胞毒性和生物活性成分對半胱天冬酶 -3 酶的分子對接分析。食品生物化學雜誌, 2021, 45(5). DOI : 10.1111/jfbc.13720。