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Research Article

An *In Silico* Study of Examining Bioactive Compounds from *Azadirachta indica* Juss. (Neem) as Potential Death Receptor 5 Inductor in Hepatoma Cells

Ricadonna Raissa[1], Anna Safitri[2,3], Masruri Masruri[2], Ma Asuncion Guiang Beltran[5], Aulanni'am Aulanni'am[2,4,*]

ABSTRACT

Hepatocellular carcinoma is a disease that occurs due to the uncontrolled growth of abnormal hepatocytes. While cancer cells will not die by itself, due to resistance to death receptors 5 (DR5)-mediated apoptosis. This study is aimed to investigate *Azadirachta indica* Juss. leaves compound, such as gedunin and nimbolide, in binding DR5 and stimulated the TNF-related apoptosis inducing ligand (TRAIL), native ligand binding to DR5, which has a role of pro-apoptotic by docking simulation. The ligand and protein preparations were done using Discovery Studio 2016 and Hex 8.0.0 for docking. Visualization was done using Discovery Studio 2016. The docking studies revealed that nimbolide has a lower binding energy with the DR5-TRAIL complex than gedunin. According to the findings, nimbolide is a more effective DR5-TRAIL binding inducer than gedunin and has a higher binding affinity for DR5-TRAIL. This interaction has the potential to significantly reduce DR5-TRAIL binding resistance. Nimbolide and gedunin can be considered as drugs that can sensitize TRAIL binding to DR5 and increase the activation of one of hepar cancers signaling apoptosis pathways.

Key words: *Azadirachta indica* Juss., Cancer, Death receptor 5, Apoptosis, *In silico*.

Ricadonna Raissa[1], Anna Safitri[2,3], Masruri Masruri[2], Ma Asuncion Guiang Beltran[5], Aulanni'am Aulanni'am[2,4,*]

[1]Doctoral Program of Chemistry, Faculty of Mathematics and Natural Science, Universitas Brawijaya, Malang, INDONESIA.
[2]Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Brawijaya, Malang, INDONESIA.
[3]Research Center for Smart Molecules of Natural Genetic Resources (SMONAGENES), Universitas Brawijaya, Malang, INDONESIA.
[4]Faculty of Veterinary Medicine, Universitas Brawijaya, Malang, INDONESIA.
[5]College of Veterinary Medicine, Tarlac Agricultural University, Camiling, Tarlac, PHILIPPINES.

Correspondence

Aulanni'am Aulanni'am

Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Brawijaya, Faculty of Veterinary Medicine, Universitas Brawijaya, Malang, INDONESIA.

E-mail: aulani@ub.ac.id

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the major contributor to cancer deaths. In 2025, HCC cases are estimated to be about >1 million cases.[1] Predilection of HCC is formed by various factor such as viral infection with hepatitis B or C viruses (HCV), cirrhosis induced by alcohol consumption and other factor.[2] Chronic HCV or cirrhosis lead abnormal proliferation of primary hepatic.[3,4] The liver cancer progression menchanism is cell proliferation. The liver cancer cells keep the abnormal proliferation by defense mechanism to apoptosis.[5]

Death receptor 5 (DR5) is pro-apoptotic protein member of the tumor necrosis factor (TNF) receptor superfamily, localized in cell surface.[6] DR5 bind to its ligand, TNF-related apoptosis inducing ligand (TRAIL) to activate the extrinsic apoptotic pathway.[7] This apoptosis mechanism of DR5-TRAIL binding potential has been the focus of attention, due to selectivity to kill the cancer cell not the normal one.[8] An *in vitro* study show that there is down-regulation expression of DR5 in cancer cells (Elrod et al. 2010). The cell cancer has a defense mechanism against the apoptosis by resisting DR5 to bind the TRAIL.[9,10] Therefore, compounds that can stimulate sensitivity DR5 binding to TRAIL is need to be explored.

Plant derived compounds are promising anti cancer therapies by apoptosis.[11] *Azadirachta indica* Juss. (Neem) plants have been used in ancient herbal medicine for the treatment of various diseases, particularly cancer.[12,13] Compounds such as gedunin and nimbolide have anticancer properties.[14–17] Gedunin and nimbolide, neem limonoid, are one of the main chemical compounds found in the neem tree [14,18–20] Recent studies have shown that gedunin can inhibit the progression of cancer cell proliferation.[21–23] Meanwhile, an *in vitro* study showed that nimbolide induction of growth arrest and apoptosis.[19,24]

In the current study, we used in silico molecular docking method to analyze the interactions between the DR5-TRAIL as an important role in hepatoma cell and neem compounds to induce apoptosis.

MATERIALS AND METHODS

Ligands preparation

The chemical compound of mangosteen which consisted of gedunin (CID: 12004512) and nimbolide (CID: 12313376) were obtained from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in SDF format. Ligands energy were minimized and converted from SDF to PDB format by BIOVIA Discovery Studio Dassault systemes® 2019 (Figure 1).

Protein preparation

The protein structure was obtained from Protein Data Bank (PDB) (https://www.rcsb.org/) as a DR5-TRAIL complex (PDB ID: 1D4V) (Figure…). The protein was then prepared for removing the ligands and water molecules by using BIOVIA Discovery Studio Dassault systemes® 2019.

Chemical interaction and 3D molecular visualization

Docking was conducted by HEX 8.0.0 software to predict the binding energy and possible ligand interactions and its receptor.[25] In this project, we

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Table 1: Molecular interaction of DR5 amino acid residues among ligands.

Interaction	Name	Distance (Å)	Category	Type	Energy (kcal/mol)
DR5 (A) – TRAIL (B)	B:ARG121:NH2 - A:ASP90:OD1	4,15562	Electrostatic	Attractive Charge	-846,53
	B:ARG158:NH2 - A:GLU89:OE2	3,73783	Electrostatic	Attractive Charge	
	B:ARG227:NH1 - A:ASP120:OD2	4,15265	Electrostatic	Attractive Charge	
	B:ARG227:NH1 - A:GLU123:OE2	4,45712	Electrostatic	Attractive Charge	
	A:ARG115:HH11 - B:GLU271:O	2,05744	Hydrogen Bond	Conventional Hydrogen Bond	
	A:ARG115:HH21 - B:GLU271:O	1,66691	Hydrogen Bond	Conventional Hydrogen Bond	
	A:SER121:HG - B:ASN228:O	2,68742	Hydrogen Bond	Conventional Hydrogen Bond	
	B:GLN208:HN - A:GLU151:OE1	2,46377	Hydrogen Bond	Conventional Hydrogen Bond	
	B:LYS224:HZ1 - A:ASP148:O	2,65532	Hydrogen Bond	Conventional Hydrogen Bond	
	B:ARG227:HH21 - A:ARG118:O	2,2749	Hydrogen Bond	Conventional Hydrogen Bond	
	A:GLU151:OE1 - B:TYR209	4,85661	Electrostatic	Pi-Anion	
	A:LEU110:CD1 - B:PHE278	3,53824	Hydrophobic	Pi-Sigma	
	A:LEU111:CD2 - B:PHE163	2,98737	Hydrophobic	Pi-Sigma	
	A:LEU114:CD1 - B:TYR185	3,86078	Hydrophobic	Pi-Sigma	
	A:LEU114:CD2 - B:TYR183	3,15595	Hydrophobic	Pi-Sigma	
	A:ARG115:O - B:TYR243	2,17318	Other	Pi-Lone Pair	
	B:HIS125 - A:PHE112	4,73791	Hydrophobic	Pi-Pi Stacked	
	B:ALA226 - A:MET152	4,24296	Hydrophobic	Alkyl	
	B:TYR243 - A:ARG115	4,45895	Hydrophobic	Pi-Alkyl	
DR5 –Gedunin	A:ARG145:HH12 - :UNK0:O1	2,62927	Hydrogen Bond	Conventional Hydrogen Bond	-237.12
	A:TRP173:HE1 - :UNK0:O4	1,96636	Hydrogen Bond	Conventional Hydrogen Bond	
	A:ARG145:CD - :UNK0:O1	3,67468	Hydrogen Bond	Carbon Hydrogen Bond	
	:UNK0:H46 - A:CYS137:O	2,15221	Hydrogen Bond	Carbon Hydrogen Bond	
	:UNK0:H66 - A:THR135:O	2,55728	Hydrogen Bond	Carbon Hydrogen Bond	
	A:VAL136:CG1 - :UNK0	3,16122	Hydrophobic	Pi-Sigma	
	A:ARG145 - :UNK0	4,4262	Hydrophobic	Alkyl	
	:UNK0:C24 - A:PRO150	4,45623	Hydrophobic	Alkyl	
DR5 (A)-Gedunin-TRAIL (B)	B:ARG121:NH2 - A:ASP90:OD1	4,55422	Electrostatic	Attractive Charge	-868,84
	B:ARG158:NH2 - A:GLU89:OE2	4,44844	Electrostatic	Attractive Charge	
	B:ARG227:NH1 - A:ASP120:OD2	4,26184	Electrostatic	Attractive Charge	
	B:ARG227:NH1 - A:GLU123:OE2	4,50501	Electrostatic	Attractive Charge	
	B:ARG227:NH2 - A:GLU123:OE1	5,58713	Electrostatic	Attractive Charge	
	A:ARG115:HH11 - B:GLU271:O	1,97128	Hydrogen Bond	Conventional Hydrogen Bond	
	A:ARG115:HH21 - B:GLU271:O	1,39071	Hydrogen Bond	Conventional Hydrogen Bond	
	A:SER121:HG - B:ASN228:O	2,44347	Hydrogen Bond	Conventional Hydrogen Bond	
	B:LYS224:HZ1 - A:ASP148:O	2,91092	Hydrogen Bond	Conventional Hydrogen Bond	
	B:ARG227:HH21 - A:ARG118:O	2,32684	Electrostatic	Pi-Cation	
	A:GLU151:OE1 - B:TYR209	3,9936	Electrostatic	Pi-Anion	
	A:LEU110:CD2 - B:PHE278	3,23751	Hydrophobic	Pi-Sigma	
	A:LEU111:CD2 - B:PHE163	3,48296	Hydrophobic	Pi-Sigma	
	A:LEU114:CD2 - B:TYR183	3,58944	Hydrophobic	Pi-Sigma	
	A:ARG115:O - B:TYR243	2,40532	Other	Pi-Lone Pair	
	B:HIS125 - A:PHE112	5,01114	Hydrophobic	Pi-Pi Stacked	
	B:ALA226 - A:MET152	4,11703	Hydrophobic	Alkyl	
	B:TYR185 - A:LEU114	4,66379	Hydrophobic	Pi-Alkyl	
	B:TYR243 - A:ARG115	4,65331	Hydrophobic	Pi-Alkyl	
DR5 - Nimbolide	A:ARG145:HH12 - :UNK0:O6	2,71593	Hydrogen Bond	Conventional Hydrogen Bond	-247,7
	:UNK0:H61 - A:ARG145:O	1,71481	Hydrogen Bond	Carbon Hydrogen Bond	
	:UNK0:H61 - A:PRO172:O	2,01755	Hydrogen Bond	Carbon Hydrogen Bond	
	:UNK0:H64 - A:GLU146:O	2,70192	Hydrogen Bond	Carbon Hydrogen Bond	
	A:CYS139:SG - :UNK0	4,65808	Other	Pi-Sulfur	
	:UNK0 - A:TRP173	5,9592	Hydrophobic	Pi-Pi T-shaped	
	:UNK0:C34 - A:ARG145	3,88385	Hydrophobic	Alkyl	
	:UNK0:C34 - A:PRO150	4,84724	Hydrophobic	Alkyl	
	A:TRP173 - :UNK0	5,45953	Hydrophobic	Pi-Alkyl	
	:UNK0 - A:ARG145	3,45812	Hydrophobic	Pi-Alkyl	

DR5 (A) – Nimbolide – TRAIL (B)	B:ARG121:NH2 - A:ASP90:OD1	4,47138	Electrostatic	Attractive Charge	
	B:ARG158:NH2 - A:GLU89:OE2	4,10473	Electrostatic	Attractive Charge	
	B:ARG227:NH1 - A:ASP120:OD2	4,19337	Electrostatic	Attractive Charge	
	B:ARG227:NH1 - A:GLU123:OE2	4,52691	Electrostatic	Attractive Charge	
	B:ARG227:NH2 - A:GLU123:OE1	5,59371	Electrostatic	Attractive Charge	
	A:ARG115:HH11 - B:GLU271:O	2,00735	Hydrogen Bond	Conventional Hydrogen Bond	
	A:ARG115:HH21 - B:GLU271:O	1,52625	Hydrogen Bond	Conventional Hydrogen Bond	
	A:SER121:HG - B:ASN228:O	2,59169	Hydrogen Bond	Conventional Hydrogen Bond	
	B:GLN208:HN - A:GLU151:OE1	2,4509	Hydrogen Bond	Conventional Hydrogen Bond	
	B:ARG227:HH21 - A:ARG118:O	2,26908	Hydrogen Bond	Conventional Hydrogen Bond	-874,96
	A:GLU151:OE1 - B:TYR209	4,82039	Electrostatic	Pi-Cation	
	A:LEU110:CD1 - B:PHE278	3,91833	Hydrophobic	Pi-Sigma	
	A:LEU110:CD2 - B:PHE278	3,10911	Hydrophobic	Pi-Sigma	
	A:LEU111:CD2 - B:PHE163	3,28832	Hydrophobic	Pi-Sigma	
	A:LEU114:CD1 - B:TYR185	3,9988	Hydrophobic	Pi-Sigma	
	A:LEU114:CD2 - B:TYR183	3,45255	Hydrophobic	Pi-Sigma	
	A:ARG115:O - B:TYR243	2,33367	Other	Pi-Lone Pair	
	B:HIS125 - A:PHE112	4,91888	Hydrophobic	Pi-Pi Stacked	
	B:ALA226 - A:MET152	4,11085	Hydrophobic	Alkyl	

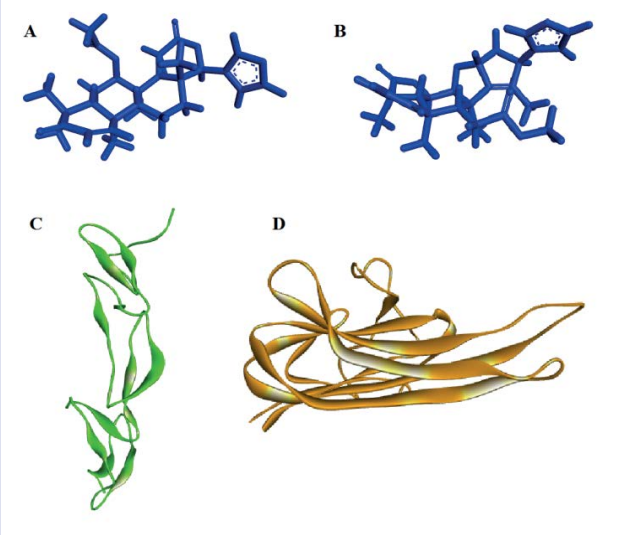

Figure 1: The chemical compound from neem: A) Gedunin (CID: 12004512); B) Nimbolide (CID: 12313376); C) death receptor 5 (DR5) (PDB ID: 1D4V); D) tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (PDB ID: 1D4V).

established several interactions such as DR5-TRAIL, DR5-gedunin. DR5-nimbolide, DR5-gedunin-TRAIL and DR5-nimbolide-TRAIL. The correlation type used in this docking was Shape+Electro+DARS. Docking results were then visualized using BIOVIA Discovery Studio Dassault systemes® 2019 to analyze the interactions.

RESULTS AND DISCUSSION

TRAIL interacted on DR5 amino acid residues includes Glu 89, Asp90, Leu110, Leu111, Leu114, Phe112, Arg115, Arg118, Asp120, Ser121, Glu123, Glu127, Asp148, Glu151, Phe152 and Asn228 with total binding energy -846,53 kcal/mol (Table 1).

Interaction between DR5 and gedunin established 4 hydrogen bonds and 3 hydrophobic bonds with total binding energy -237.12 kcal/mol (Table 1). Those interactions were bound with 5 amino acid residues such as Thr135, Cys137, Arg145, Pro150, and Trp173 outside of the DR5-TRAIL binding site (Figure 2). The DR5-gedunin complex interacted with TRAIL had total binding energy of -868.84 kcal/mol (Table 1). This complex may enhance DR5 activity by forming 4 hydrogen bonds in Arg115, Ser11 and Lys224 and one electrostatic interaction in Glu151 which are the active site of DR5 (Figure 2).

Nimbolide interacted with 4 amino acid residues DR5 with total binding energy -247.7 kcal/mol (Table 1). Nimbolide forms hydrogen bonds with Arg145, Glu146 and Pro172 which indicate that there is a strong bond established between the ligand and active site of DR5 located in Domains II (Figure 2). TRAIL, which interacted with the DR5-nimbolide complex had – 874.96 kcal/mol of total binding energy.

A hydrogen bond was formed in Arg115, Arg118, Ser121, and Glu151 at the active site of DR5 (Figure 3). There are two electrostatic interactions in Glu151, twice the gedunin binding. Electrostatic interactions with each other to form a stable binding.[26] Just like the DR5-gedunin-TRAIL complex, this complex may enhance DR5 activity. The thread of nimbolide before gedunin treatment showed the best result for compound combination to enhance DR5-TRAIL binding.

DR5 is a member of the tumor necrosis factor (TNF) receptor superfamily. DR5 has cytoplasmic death domains to induce cell apoptosis. The extracellular domain of the receptors is characterized by the concatenated cysteine-rich domains (CRDs) that are responsible for ligand binding. DR5 forms a multimer that activates the extrinsic apoptotic pathway.[27,28] Inspection of the DR5 sequences shows in Domain 1 (D1) and in Domain 2 (D2). D2 is implicated as a major focus for TRAIL-binding specificity, with conservation. Domain 2 starts from amino acid 137 until 179.[29]

TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) is the native ligand of receptor DR5, a cytokine that preferentially induces apoptosis in tumor cells compared with normal cells through two receptors (DR4 and DR5).[9,30] TRAIL has selective induction of apoptosis in malignant cells *via* its receptor.[2] TRAIL attracts great research interest for its selective induction of apoptosis in malignant cells *via* its receptors, DR5.[31] The resistance TRAIL to bind the DR5 implicate in a variety of human cancers such as hepatocarcinoma cells.[32]

Lots of studies have evaluated the anti-cancer activity of nimbolide and gedunin. Recent studies have shown that gedunin can inhibit the proliferation of cancer cells including those of the prostate, ovary, and colon.[21,22] Previous studies showed that gedunin and nimbolide increase the occurrence of apoptosis in cell cancer.[23,33] Nimbolide inhibits a number of survival proteins, and upregulates the death receptors (DR) that interact with TRAIL, namely DR4 and DR5. The combined effects of nimbolide's actions increase the apoptotic consequences of TRAIL therapy.[14,18]

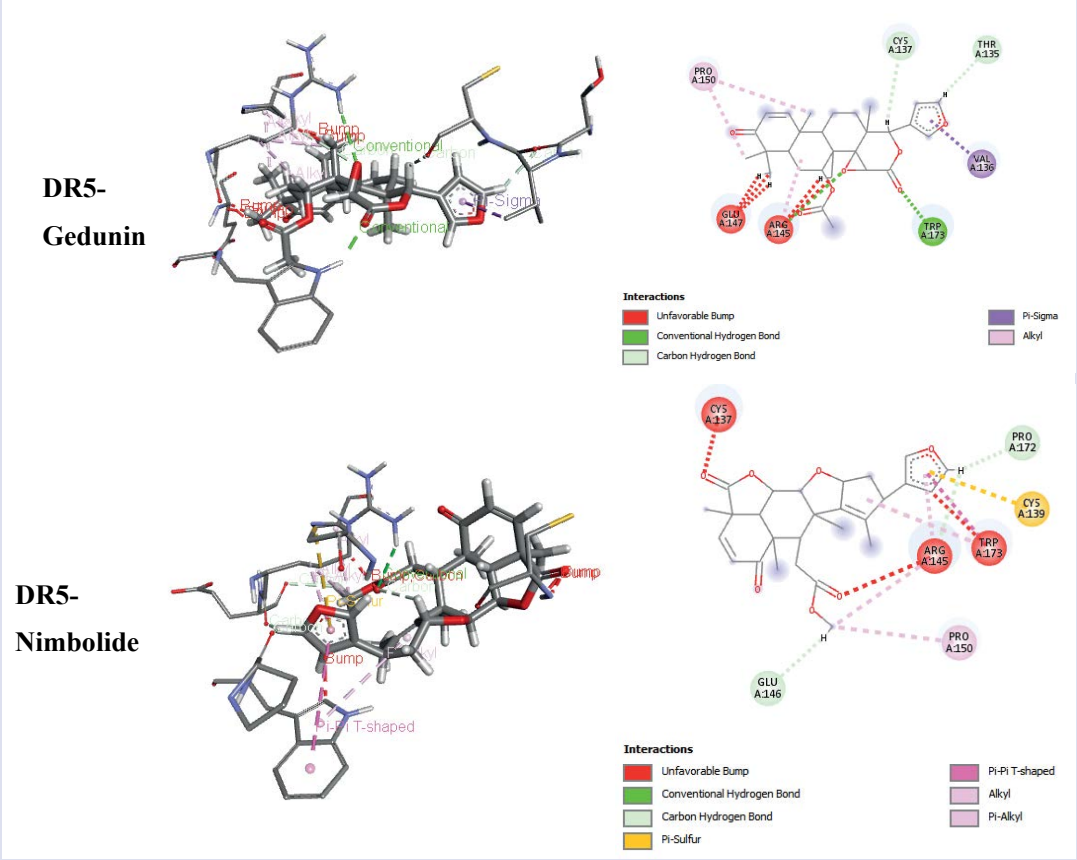

Figure 2: DR5 binding with gedunin and nimbolide in 2D diagram ligand interaction.

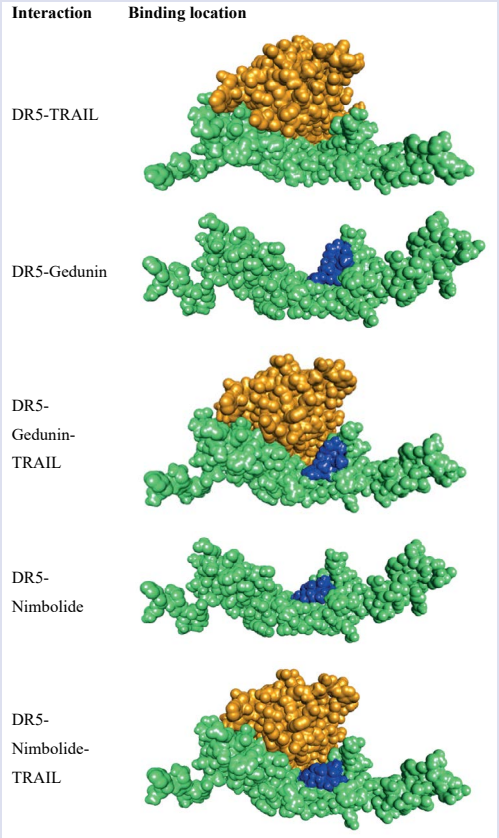

Figure 3: Binding location and interaction DR5 and ligands (DR5: green; TRAIL (native ligand): yellow; ligand: blue).

This study showed that gedunin, nimbolide and a combination of TRAIL can bind in the active site of DR5 which means they can enhance DR5 to bind with its ligands. Nimbolide and gedunin might increase the binding affinity between DR5 and TRAIL. Both nimbolide and gedunin decrease binding energy when DR5 is docked with TRAIL. Although gedunin was not bound in the active site of DR5-TRAIL, the binding site is still in the DR5 Domain II. Nearly all of these interactions bind the DR5 Domain II in amino acids 137-179 which shows that TRAIL binds to the DR5 Domain II participating in ligand binding. Domain 2 is implicated as a major focus for DR5-TRAIL binding specificity.[2] These results indicate gedunin and nimbolide had the stable potential binding to DR5.

The docking results showed that nimbolide has lower binding energy than gedunin with DR-TRAIL complex. The data indicated that nimbolide has a higher potential DR5-TRAIL binding inducer than gedunin and a stronger binding affinity with DR5 (Table 1). The lower binding energy indicates more stable binding between the molecules than the molecule with higher binding energy.[34] This interaction may potentially reduce the resistance of DR5-TRAIL binding.

When TRAIL binds to its receptor, DR5, it causes the receptor to trimerize and the intracellular death domain (DD) of the receptor to cluster, resulting in the development of the death-inducing signaling complex (DISC). The recruitment of FAS-associated death domain protein (FADD), and subsequent binding and activation of caspase-8 and -10. Activated caspase-8 and -10 then cleaved caspase-3, causing the death substrates to be cleaved.[35] However, their potential is stills need to be examined through further analysis to uncover the further potential.

CONCLUSION

In summary, bioactive compounds from neem, such as gedunin and nimbolide have potential as inhibitors of the interaction between DR5 and TRAIL as native ligands. These two compounds were proven to bind with DR5 in their ligand binding site. Nimbolide had shown the best results among other complexes that were tested with threads of nimbolide before TRAIL. Gedunin and nimbolide can be considered as drugs that can sensitize TRAIL binding to DR5 and increase the activation of one of hepar cancers signaling apoptosis pathways.

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DISCLOSURE STATEMENT

The authors declare no conflicts of interests.

ABBREVIATIONS

Caspases: Cysteine-aspartic proteases; CID: PubChem Compound Identifier; CRDs: Concatenated cysteine-rich domains; DD: Death domain; DISC: Death-inducing signaling complex; DR: Death receptor; DR5: Death receptor 5; HCC: Hepatocellular carcinoma; HCV: Hepatitis B or C viruses; PDB: Protein Data Bank; TNF: Tumor necrosis factor; TRAIL: TNF-related apoptosis inducing ligand.

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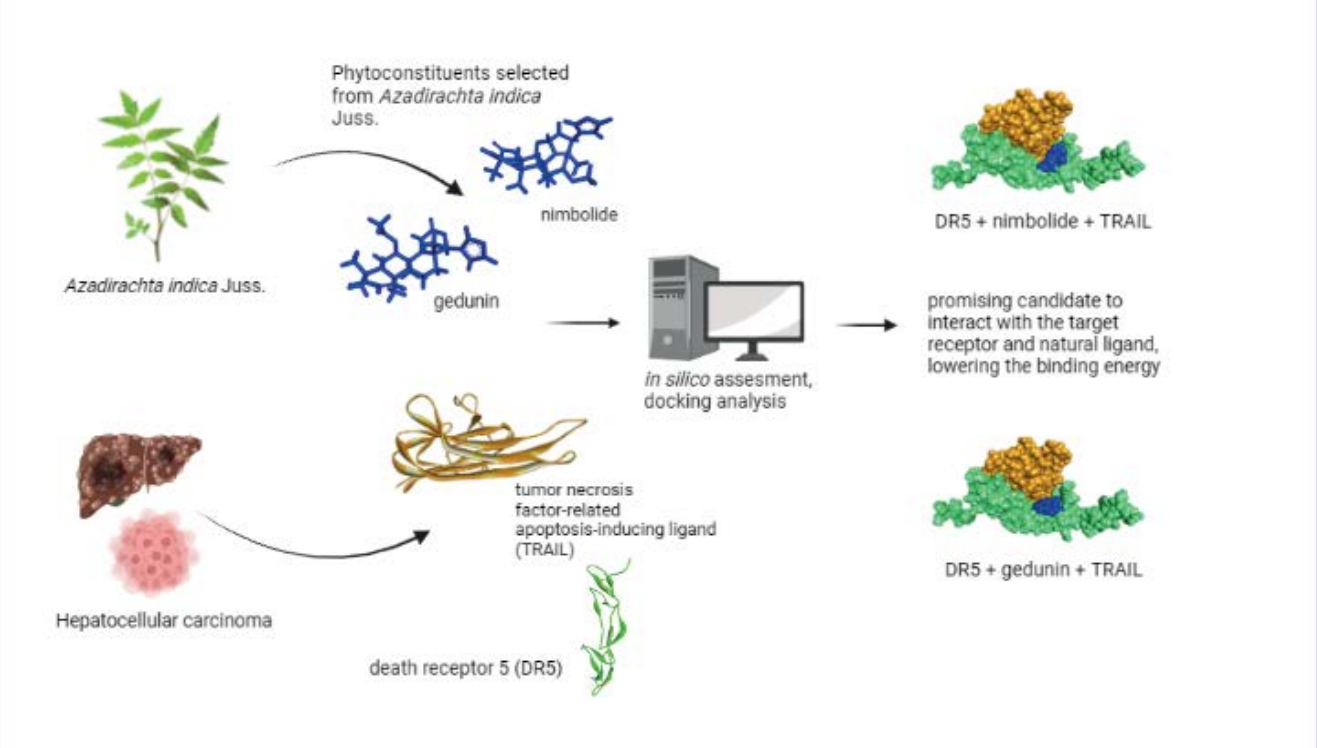
GRAPHICAL ABSTRACT



ABOUT AUTHORS

Ricadonna Raissa is a Doctoral Candidate in Chemistry at Universitas Brawijaya, Indonesia. She completed her DVM. in Veterinary Medicine at Universitas Airlangga, Indonesia and M.Sc. in Chemistry (Biochemistry) at Universitas Brawijaya, Indonesia. Currently, she is an awardee of the PMDSU Scholarship (Batch IV) at Universitas Brawijaya, Indonesia. Her research projects are related to cancer, bioinformatics, and molecular biology. His actual research focus is the application of traditional medicine plants to treat as anticancer in Indonesia.

Aulanniam Aulanniam, a Professor of Biochemistry in Universitas Brawijaya, she completed her DVM. in Veterinary Medicine at Universitas Airlangga, Indonesia, master degree DES in Molecular Biochemistry at USTL-Montpellier France, and Doctoral in Medicine Biochemistry at Universitas Airlangga, Indonesia. Her research interests on the molecular enzymology, biopeptide, protein complexes, medical devices, autoimmunee diseases and immunocontraception. The author has published more than 100 manuscripts in Scopus-indexed journals.

Ma Asuncion Guiang Beltran, is a Professor of Veterinary Medicine in Tarlac Agricultural University, Philippine, master degree in Tropical Veterinary Medicine at James Cook University, Australia, and Doctoral in Animal Science at Central Luzon State University (CLSU), Philippine. Her research interests on the biomedical sciences. The author has published more than 100 manuscripts in Scopus-indexed journals.

Anna Safitri, is an academic staff in Chemistry Department, Brawijaya University, also a member of Smart Molecule of Natural Genetics Resource (SMONAGENES). She received Master and PhD from School of Chemistry, The University of Sydney, Australia. Her research interests include development drug design from plants, biochemistry, and molecular biology. The author has published more than 30 manuscripts in Scopus-indexed journals.

Masruri Masruri, is an Assistant Professor in Chemistry, Brawijaya University. He received master degree in Chemistry from Universitas Gajah Mada, Indonesia and doctoral degree Ph.D in Chemistry from Australian national University. His research interest include synthetic organic chemistry, organic chemistry and natural product chemistry. The author has published more than 50 manuscripts in Scopus-indexed journals.

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