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**ORIGINAL RESEARCH PAPER**

# EVALUATING THE MOLECULAR INTERACTION OF *SAMBUCUS* PLANT BIOACTIVE COMPOUNDS TOWARD TNF-R1 AND TRAIL-R1/R2 AS POSSIBLE ANTI-CANCER THERAPY BASED ON TRADITIONAL MEDICINE: THE BIOINFOTMATICS STUDY

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**Abstract:** Inducing the apoptosis signaling pathway is one of the favorable treatment to overcome cancer incidence. Current treatment showed the increasing usage of natural products as therapy. Thus, the present work aims to evaluate the molecular interaction of *Sambucus* plant bioactive compounds toward the TNF-R1 and TRAIL-R1/R2 as possible anti-cancer therapy through the computational study. Approximately 31 bioactive compounds of *Sambucus* plant were screened as potential ligands to bind the TNF-R and TRAIL-R1/R2 protein models. Molecular docking was performed to evaluate the interactive features of the ligands toward the apoptosis channels. In this present study, the results showed the Pro C1 was on the top with the lowest free binding energy on the interaction with TNF-R1, TRAIL-R1, and TRAIL-R2. Moreover, based on the computational prediction demonstrated that several bioactive compounds from *Sambucus* plant have similar residues in the interaction with TNF-R1, TRAIL-R1, and TRAIL-R2. The evidence from this study implied that Pro C1 that mostly found in *Sambucus* plant has therapeutic potency as the activator of apoptotic signaling pathways through the interaction with TNF-R1 and TRAIL-R1/R2.

**Keywords:** *apoptosis, computational study, Sambucus, TNF-R1, TRAIL-R1/R2*

## INTRODUCTION

The apoptosis is the life-end phase of the primary biological process of the cell. This programmed death cell often characterized by shrinking of the plasma membrane called plasmolysis, followed with the condensed and fragmented nuclei [1 – 3]. Apparently, this phenomenon has been conferred a novel insight to harnessing the cancer therapy by targeting and inducing apoptosis in cancer cells [4 – 6]. Furthermore, numerous studies have been pointed out several activation factors of apoptosis signaling pathways. The common forms of activation are triggered by the protein interaction between Fas ligand with Fas, TNF-α with TNF-R1, and TRAIL with TRAIL-R1/R2 [7, 8].

To a greater extent, the initiation of the apoptosis signaling pathway started when the TNF-α is binding with TNF-R1. Then, this binding activates the death-inducing signaling complex (DISC) which consists of TNFR-associated death domain protein (TRADD), Fas-associated protein with death domain (FADD), TNFR-associated factor 1 (TRAF1), and caspase-8 [9, 10]. In the same way, the TRAIL also induces the apoptosis signaling pathways through the interaction with TRAIL-R1/R2. The interaction then results in the DISC formation. This complex apparently recruits FADD as an adaptor molecule and continuously binding the caspase-8 and -10. Finally, apoptosis occurs alongside with the cleaved of caspase-3 which was resulting from the activation of caspase-8 and -10 [11, 12].

Many years have been dedicated to evaluate the possibility of targeting TNF-α/TNF-R1 and TRAIL/TRAIL-R1/R2 apoptosis signaling pathway as the solution for cancer therapy, one of them is utilizing or optimizing the natural products [9]. The study conducted by Tang et al., (2012) demonstrated that Casticin, a biochemical compound isolated from *Vitex rotundifolia* shows the medicinal effect to promote TRAIL-induced apoptosis through decreasing the cell survival proteins and increasing the TRAIL-R2 expression [13]. Another study carried by Jin et al., (2007) unrevealed that flavonoids promote the apoptosis rates by enhancing the expression of Fas of TRAIL receptors [10].

As described above, natural products have a high possibility of being anti-cancer including *Sambucus* bioactive compounds. *Sambucus* plant belongs to the Adoxaceae family characterized by the scrub shaped plant that can grow up to 8-10 m and widely spread in the wide ecosystem [14, 15]. Several types of species of this family are *Sambucus nigra*, *Sambucus ebulus*, and *Sambucus javanica*. As one of the medicinal plant, the *Sambucus* plant contains broad bioactive compounds, such as flavonoids and phenol [14, 16]. It has been reported that several types of *Sambucus* plants have multiple therapeutic potencies including antiviral, antiinflammation, antibacterial, antioxidant, and antidiabetic [14, 16, 17]. Thus, the present study aims to evaluate the molecular interaction of *Sambucus* plant bioactive compounds toward TNF-R1 and TRAIL-R1/R2 as possible anti-cancer therapy through the computational study.

## MATERIALS AND METHODS

### Sample determination

Approximately 31 bioactive compounds were applied for docking simulation against TNF-R, TRAIL-R1/R2 protein model. The bioactive compounds used in this study as shown in table 1 referred to the respective previous study on the chemicals content of *Sambucus* plant [14, 16 – 18].

***Table 1***. *Multiple Sambucus bioactive compounds used in this study*

| No. | Bioactive Compound | Molecular Formula | CID |
|---|---|---|---|
| 1 | Protocatechuic acid | $C_7H_6O_4$ | 72 |
| 2 | 4-Hydroxybenzaldehyde | $C_7H_6O_2$ | 126 |
| 3 | Benzoic acid | $C_7H_6O_2$ | 243 |
| 4 | Hippuric acid | $C_9H_9NO_3$ | 464 |
| 5 | 3,4-Dihydroxyphenylacetic acid | $C_8H_8O_4$ | 547 |
| 6 | Naringenin | $C_{15}H_{12}O_5$ | 932 |
| 7 | Homovanillic acid | $C_9H_{10}O_4$ | 1738 |
| 8 | 3-Hydroxybenzoic acid | $C_7H_6O_3$ | 7420 |
| 9 | 4-Methylcatechol | $C_7H_8O_2$ | 9958 |
| 10 | Phloroglucinolaldehyde | $C_7H_6O_4$ | 68099 |
| 11 | Epicatechin | $C_{15}H_{14}O_6$ | 72276 |
| 12 | Catechin | $C_{15}H_{14}O_6$ | 73160 |
| 13 | Procyanidin B2 | $C_{30}H_{26}O_{12}$ | 122738 |
| 14 | Procyanidin B5 | $C_{30}H_{26}O_{12}$ | 124017 |
| 15 | Cyanidin | $C_{15}H_{11}O_6^+$ | 128861 |
| 16 | Procyanidin C1 | $C_{45}H_{38}O_{18}$ | 169853 |
| 17 | Ferulic acid | $C_{10}H_{10}O_4$ | 445858 |
| 18 | *p*-Coumaric acid | $C_9H_8O_3$ | 637542 |
| 19 | Caffeic acid | $C_9H_8O_4$ | 689043 |
| 20 | Quercetin | $C_{15}H_{10}O_7$ | 5280343 |
| 21 | Isoquercitrin | $C_{21}H_{20}O_{12}$ | 5280804 |
| 22 | Rutin | $C_{27}H_{30}O_{16}$ | 5280805 |
| 23 | Kaempferol | $C_{15}H_{10}O_6$ | 5280863 |
| 24 | Isorhamnetin | $C_{16}H_{12}O_7$ | 5281654 |
| 25 | Kaempferol-3-rutinoside | $C_{27}H_{30}O_{15}$ | 5318767 |
| 26 | Quercetin-3-rhamnoside | $C_{21}H_{20}O_{11}$ | 5353915 |
| 27 | Isorhamnetin-3-rutinoside | $C_{28}H_{32}O_{16}$ | 17751019 |
| 28 | Quercetin-3-glucoside | $C_{21}H_{19}O_{12}^-$ | 25203368 |
| 29 | Pelargonidin-3-sambubioside | $C_{26}H_{29}O_{14}^+$ | 44256622 |
| 30 | Cyanidin-3-sambubioside | $C_{26}H_{29}O_{15}^+$ | 74976920 |
| 31 | Hyperoside | $C_{21}H_{20}O_{12}$ | 133568467 |

### Ligands Retrieval and Preparation

The medicinal properties of each chemical were evaluated by PASS online prediction [19]. Then, the 2D structure of the bioactive compound of the *Sambucus* plant was

collected from PubChem website (pubchem.ncbi.nlm.nih.gov/) [20]. Importantly, the structure then saved in sdf. format for further simulation processes.

### Protein Preparation and Modelling

The protein sequences of TNF-R1 (P19438), TRAIL-R1 (O00220), and TRAIL-R2 (O14763) were obtained from UniProtKB (uniprot.org/) [21]. SWISS-MODEL (https://swissmodel.expasy.org/) then used to build the protein structure [22] of TNF-R1 (1ncf.1.B), TRAIL-R1 (5 cir.1.D), and TRAIL-R2 (1 du 3.1.B). The proper designed-proteins then saved in pdb. format for further processes.

### Molecular Docking and Visualization

In this present study, the polarity compensation was set up to optimize the ligands. After that, the ligands energy minimization was applied by using Open Babel GUI and then directly converted to pdbqt. format. Blinded docking were applied to see the possible binding interaction of the ligands to the whole protein binding site. The docking simulation and prediction among the ligands to the protein models were done by occupying AutoDock Vina in PyRx 0.8. [23]. This software simultaneously allowed the ligands to interact with several possible binding site of the protein. Finally, the results were analyzed and visualized by using Pymol and ADS Visualizer software as same as the previous study [24, 25].

## RESULTS AND DISCUSSION

TNF-α/TNF-R1 binding activates has a broad spectrum of cellular responses including cell proliferation, inflammation and apoptosis [9]. Several receptors under tumor necrosis factor (TNF) receptor family have been known as death signaling channels characterized with death domain. Previously, there are five death receptor that been explored such as TNF-R1, TRAMP, TRAIL-R1, TRAIL-R2, and Fas. However, from all of these death receptors, it was reported that TRAIL can induce rapid apoptosis and is distributed in a broad type of tissue. The apoptosis signaling is induced by TRAIL through its heteroreceptor, TRAIL-R1 (DR4) and TRAIL R-2 (DR5) [8] In this preliminary study, several characteristics, behavior, and ability of *Sambucus* chemical compounds interaction toward the apoptosis channel TNF-R1 and TRAIL-R1/R2 were evaluated (Figure 1). Thus, logically from its interaction and protein activation cascade can promote the apoptosis signaling pathway in the cancer cell.

***Table 2.*** *Top three highest binding affinity of protein and ligands interaction*

| No | Protein | Ligand | Binding Affinity [kcal·mol$^{-1}$] | Amino Acid Residues |
|---|---|---|---|---|
| 1 | TNF-R1 | Procyanidin C1 | -7.7 | **ALA91, SER103, ARG121, ASP122, THR123, VAL124, CYS125, TYR135, GLU138, ASN139, PHE141, LYS161**, ILE114, ARG133, SER137, GLU160 |
| | | Isorhamnetin-3-rutinoside | -7.3 | **ALA91, SER103, ARG121, ASP122, THR123, VAL124, CYS125, TYR135, GLU138, ASN139, PHE141, LYS161**, PHE89, LEU100, ARG131 |
| | | Procyanidin B2 | -7.2 | **ALA91, SER103, ARG121, ASP122, THR123, VAL124, CYS125, TYR135, GLU138, ASN139, PHE141, LYS161**, PHE89, ARG106, ARG133 |
| 2 | TRAIL-R1 | Procyanidin C1 | -8.1 | **THR168, LYS171, GLU174, ALA187, CYS188, GLN189, CYS190, ARG196, ASP198, ALA201, TRP224**, TYR154, LEU165, PRO166, ARG177, ASN185, GLU202 |
| | | Procyanidin B2 | -7.9 | **THR168, LYS171, GLU174, ALA187, CYS188, GLN189, CYS190, ARG196, ASP198, ALA201, TRP224**, ASN185, THR186, ASN197, GLU202, PRO223 |
| | | Isorhamnetin-3-rutinoside | -7.8 | **THR168, LYS171, GLU174, ALA187, CYS188, GLN189, CYS190, ARG196, ASP198, ALA201, TRP224**, CYS170, ARG177, THR186, ASN197 |
| 3 | TRAIL-R2 | Procyanidin C1 | -8.3 | **THR117, GLU123, THR135, VAL136, CYS137, GLN138, ARG145, GLU146, GLU147, PRO150, GLU151, TRP173**, TYR103, LEU114, LEU126, ASN134, CYS139, PRO172 |
| | | Rutin | -8.0 | **THR117, GLU123, THR135, VAL136, CYS137, GLN138, ARG145, GLU146, GLU147, PRO150, GLU151, TRP173**, ASP120, LEU126, MET152 |
| | | Procyanidin B2 | -7.9 | **THR117, GLU123, THR135, VAL136, CYS137, GLN138, ARG145, GLU146, GLU147, PRO150, GLU151, TRP173**, ASP120, ASN134, CYS139, MET152, PRO172 |
| | | Isorhamnetin-3-rutinoside | -7.9 | **THR117, GLU123, THR135, VAL136, CYS137, GLN138, ARG145, GLU146, GLU147, PRO150, GLU151, TRP173**, ASP120, LEU126, ASN134 |

In this present study, the results showed the Pro C1 was on the top with the lowest free binding energy on the interaction with TNF-R1, TRAIL-R1, and TRAIL-R2 (Table 2). The lowest free binding energy indicates the Pro C1 has the greatest ability to interact effectively with the receptors. Moreover, based on the computational prediction demonstrated that several bioactive compounds have similar residues in the interaction with TNF-R1, TRAIL-R1, and TRAIL-R2 (Table 2). These similar amino acid residues indicate that the ligands are almost in the same coordinate (Figure 2).

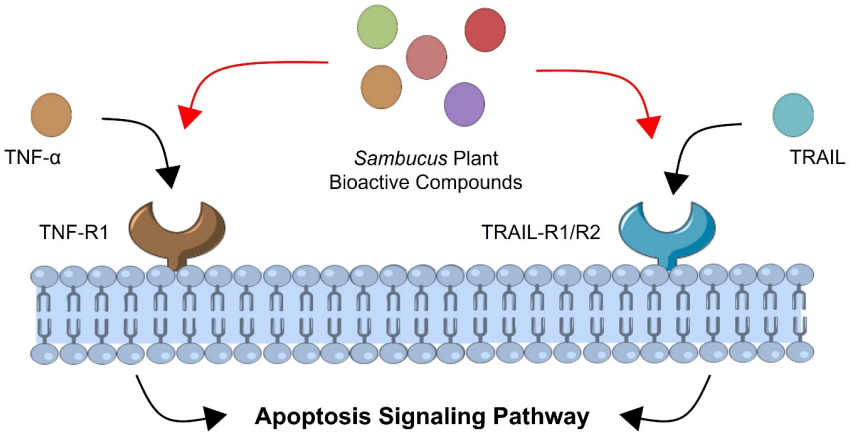


***Figure 1.*** *The schematic diagram illustrates Sambucus plant bioactive compounds may interact toward the TNF-R1 and TRAIL-R1/R2 to promote apoptosis signaling pathway*

Several bioactive compounds such as flavonoids and isoflavonoids have demonstrated large biological effects ranging from the suppression of cell cycle, cell proliferation, and oxidative stress and the promotion of detoxification enzymes, apoptosis, and immune system in which these compounds addressed to applied in cancer prevention [26 – 28]. Procyanidins are the part of proanthocyanidins which consist of flavan-3-ol units [29, 30]. Procyanidins widely distributed within the fruits, especially the berries group [29 – 31]. Also, it has been reported that the proanthocyanidins have wide biological activities like anti-carcinogenic, anti-inflammatory, anti-diabetic, anti-angiogenic, anti-oxidant, anti-allergic, cardio-protective, and anti-asthmatic [29, 32].

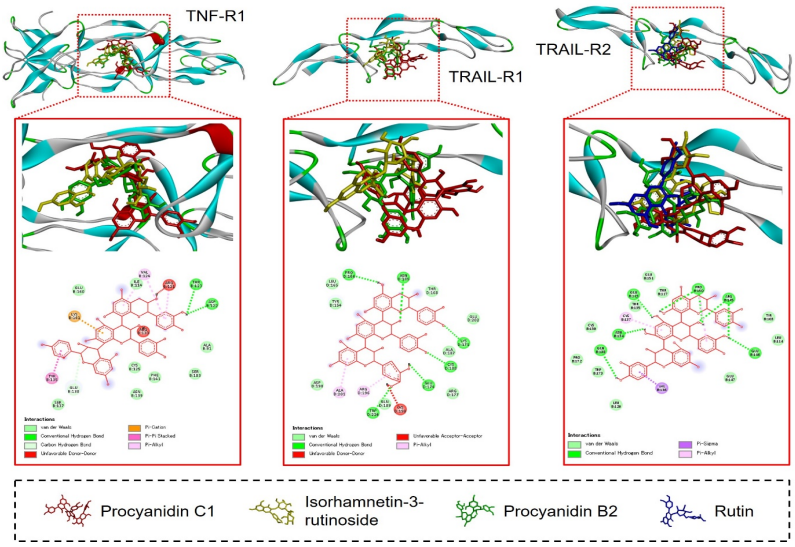


***Figure 2.*** *Protein-ligands interaction. The amino acid residues were bound to the ligand with particular binding. Left panel – TNF-R1 interacts with procyanidin C1, isorhamnetin-3-rutinoside, or procyanidin B2. Middle panel – TRAIL-R1 interacts with procyanidin C1, isorhamnetin-3-rutinoside, or procyanidin B2. Right panel – TRAIL-R2 interacts with procyanidin C1, isorhamnetin-3-rutinoside, procyanidin B2, or rutin*

To a greater extent, besides the binding affinity and amino acid residues, the other features of protein-ligand interaction can be widely observed such as hydrophobicity, hydrogen bonds, interpolated charge, and ionizability (Figure 3). Importantly, hydrophobic interaction has critical role in maintaining the formation and stability of molecular interactions and biological structures. Thus, hydrophobic interaction has been immersed as a tool to understanding various crucial biological activities like protein folding and protein-ligand binding [33]. According to Patil et al., (2010) some of weak intermolecular interactions such as hydrophobic interaction and hydrogen bonds are necessary for interaction stabilization [34].

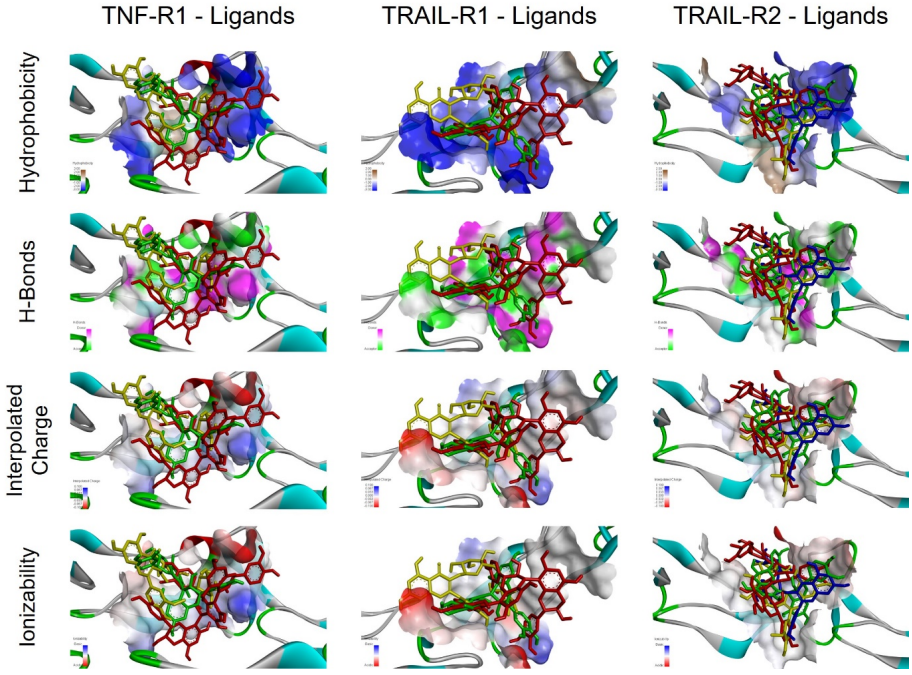


***Figure 3.*** *Molecular interaction features on TNF-R1 and TRAIL-R1/R2 with Sambucus bioactive compound are including hydrophobicity, hydrogen bonds, interpolated charge, and ionizability. Left panel - TNF-R1 interacts with procyanidin C1, isorhamnetin-3-rutinoside, or procyanidin B2. Middle panel - TRAIL-R1 interacts with procyanidin C1, isorhamnetin-3-rutinoside, or procyanidin B2, Right panel - TRAIL-R2 interacts with procyanidin C1, isorhamnetin-3-rutinoside, procyanidin B2, or rutin*

## CONCLUSION

The evidence from this study implies that Pro C1 that mostly found in *Sambucus* plant has therapeutic potency as the activator of apoptotic signaling pathways through the interaction with TNF-R1 and TRAIL-R1/R2. However, a further experimental investigation needs to be performed to prove this computational prediction.

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