IN SILICO STUDY DEMONSTRATES MULTIPLE BIOACTIVE COMPOUNDS OF *Sambucus* PLANT PROMOTE DEATH CELL SIGNALING PATHWAY VIA FAS RECEPTOR

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Abstract: In recent times, *in silico* analysis is one of the powerful tools to screen and predict the pharmaceutical properties of multiple small molecules. Furthermore, in this present study, we attempt to figure out the agonist activity of *Sambucus* bioactive compounds such as oleanic acid, ursolic acid, sambunigrin, and catechin toward tumor necrosis factor receptor superfamily member 6 (Fas). To achieve these objectives, 3D protein structure of Fas was designed by SWISS-MODEL, and the 2D structure of bioactive compounds was retrieved from PubChem. Molecular docking and visualization were done by using PyRx and Accelrys Discovery Studio Visualizer software respectively. The *in silico* study showed that *Sambucus* bioactive compounds including ursolic acid, oleanic acid, sambunigrin, and catechin have a high possibility to interact toward Fas with following binding affinity -6.6, -6.5, -6.2, and -6.1 kcal/mol. Moreover, the same amino acid residues were found in the particular protein-ligand interaction. Specifically, the amino acid residues that bind with ligands, namely Fas-ursolic acid (LYS251, ASP297, ALA301, THR241, CYS304, THR305, GLU308, PHE248, GLN244, GLY247), Fas-oleanic acid (LYS251, ASP297, ALA301, THR241, CYS304, THR305, LYS300, PHE248, GLN244, SER243), Fas-sambunigrin (LYS251, ASP297, ALA301, ASN252, THR293, LYS296, LYS300, PHE248, GLN244, GLY247), and Fas-catechin (LYS251, ASP297, ALA301, ASN252, THR293, LYS296, LYS300). Finally, the hydrophobicity, hydrogen bonds, and interpolated charge governed the binding affinities among the ligands and the Fas as a targeted receptor. Therefore, it suggests that the *Sambucus* bioactive compounds have pro-apoptotic activity through inducing death cell extrinsic signaling pathway via Fas.

Keywords: Apoptosis, bioactive compounds, Fas, *in silico*, *Sambucus* plant

Introduction

Numerous scientific types of research and discoveries pay more attention to understanding cancer and its therapies. The cancer is a group of deathly diseases which characterized by abnormal growth and unstoppable proliferation (Hu and Fu, 2012; Mantovani *et al.*, 2008). Statistically, in the United States, the number of estimated death caused by cancer in 2018 approximately 609,640 cases, and the estimated new incidence is predicted about 1,735,350 cases (Siegel *et al.*, 2018). Recently, many reports assessed multiple possible factors that might reduce cancer progression, one of them is inducing apoptosis signaling pathway (O'Brien *et al.*, 2005).

Generally, the apoptosis signaling pathway consists of two mechanisms, i.e., intrinsic manner and extrinsic manner. Particularly, intrinsic pathway is related to metabolism that fully controlled by mitochondria. Whereas the extrinsic pathway is caused by interaction and activation of death-cell receptors by its ligands, for instance, the interaction between Fas and Fas ligand (FasL) which induced the apoptosis signaling pathway (Villa-Morales and Fernández-Piqueras, 2012). Not to mention, Fas/FasL interaction is favorable targeted therapy to suppress the cancer progression through inducing apoptosis (Peter *et al.*, 2015).

The current trend shows a positive impact on that the application of traditional medicine against numerous diseases (Ekor, 2013). *Sambucus* is a group of plants which possessed medicinal properties that have been utilized for a long time for a food source and traditional medicine. It has been noted that *Sambucus* has great potential as anti-viral, anti-bacteria, anti-influenza, and anti-diabetes (Badescu *et al.*, 2015; Barsett *et al.*, 2012; Barak *et al.*, 2002). Interestingly, *Sambucus* plants contain bioactive-rich compounds such as oleanic acid, ursolic acid, sambunigrin, sambucin, chrysanthemin, and catechin (Ho *et al.*, 2017; Kamenjaković *et al.*, 2017; Barsett *et al.*, 2012). Though it has much pharmaceutics beneficial effect but its effect on apoptosis not been studied comprehensively. From above explanation, therefore, promoting the use of traditional medicine as a health product is necessary due to its several advantageous such as abundant sources, fewer side effects, and economically achieved (Mahomoodally, 2013; Pan *et al.*, 2013). In this present study, the agonist activity of *Sambucus* bioactive compounds toward Fas was evaluated in order to induce the apoptosis signaling pathway.

Materials and Methods

Ligands retrieval and preparation

In this study, several bioactive compounds that extensively found in *Sambucus* plants; they are oleanic acid, ursolic acid, sambunigrin, and catechin (Table 1). The 2D structure of those compounds was retrieved from PubChem (http://pubchem.ncbi.nlm.nih.gov/) (Kim *et al.*, 2016). Then, each compound was evaluated to discern between a drug like and non-drug like molecules based on Lipinski's rule of five (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp) (Kalidasu and Kuna, 2012). Anti-cancer activity of each bioactive compound was assessed by using PASS online prediction (http://www.pharmaexpert.ru/passonline/) (Stepanchikova *et al.*, 2003).

Table 1: Several bio-active compounds that abundantly found in elderberry plants

S/N	Chemical Name	Molecular Formula	CID	PubChem Website
1	Oleanic acid	$C_{30}H_{48}O_3$	10494	https://bit.ly/2S0vKFF
2	Ursolic acid	$C_{30}H_{48}O_3$	64945	https://bit.ly/2PGanYk
3	Sambunigrin	$C_{14}H_{17}NO_6$	91434	https://bit.ly/2P8QPyO
4	Catechin	$C_{15}H_{14}O_6$	73160	https://bit.ly/2ymAnl0

Protein preparation and modeling

The protein sequence of tumor necrosis factor receptor superfamily member 6 (ID: **P25445-1**) were retrieved from UniProtKB (www.uniprot.org) (Chen *et al.*, 2017). After that continued by protein 3D modeling (3ezq.1.A) via SWISS-MODEL (www.swissmodel.expasy.org) (Waterhouse *et al.*, 2018).

Molecular docking and visualization

In this study, protein and ligands were optimized by adding polarity. Then, ligand energy minimization was achieved via

Open Babel GUI which was integrated with PyRx and converted to pdbqt format. Molecular docking was accomplished via AutoDock Vina in PyRx 0.8 (https://pyrx.sourceforge.io/) (Trott and Olson, 2010). Molecular docking results were analyzed and visualized by using Accelrys Discovery Studio Visualizer software (http://accelrys.com/) as like as previous study (Putra *et al.*, 2017).

Results and Discussion

A strong understanding of protein interaction toward small molecules provides evidence about protein function, characteristic, and its possible therapeutic intervention for medical purposes. Therefore, this study attempt to understand how *Sambucus* bioactive compounds can be alternative molecules that induce death cells extrinsic signaling pathway by interacting with Fas (Fig. 1). According to Ramos (2007), flavonoids mediated the apoptosis signaling pathway by activating several numbers of a crucial element in signal transduction pathways. Furthermore, another report described the flavonoids promote activation of caspase-2, caspase-3, and caspase-9 in rat hepatoma cells (Wätjen *et al.*, 2005).

In silico analysis in the field of bioactive compounds screening is helpful to generate models of biological activity and physicochemical properties (Ejeh *et al.*, 2017). As noted, it was found that the bioactive compounds such as ursolic acid, oleanic acid, sambunigrin, and catechin bind with the Fas in a proper manner (Fig. 2). More specific, binding affinity analysis also showed the certain number in which the compounds need minimum energy to bind with the receptor (Table 2). Sequentially, Fas bind to ursolic acid, oleanic acid, sambunigrin, and catechin with minimum energy binding -6.6, -6.5, -6.2, and -6.1 kcal/mol, respectively. These findings suggest that the above *Sambucus* bioactive compounds have potential possibility to bind and interact with Fas. Moreover, there are a lot of factors that change the binding energy such as ionization effects, electrostatic and van der Waals interactions, conformational changes and the role of solvent (Perozzo *et al.*, 2004).

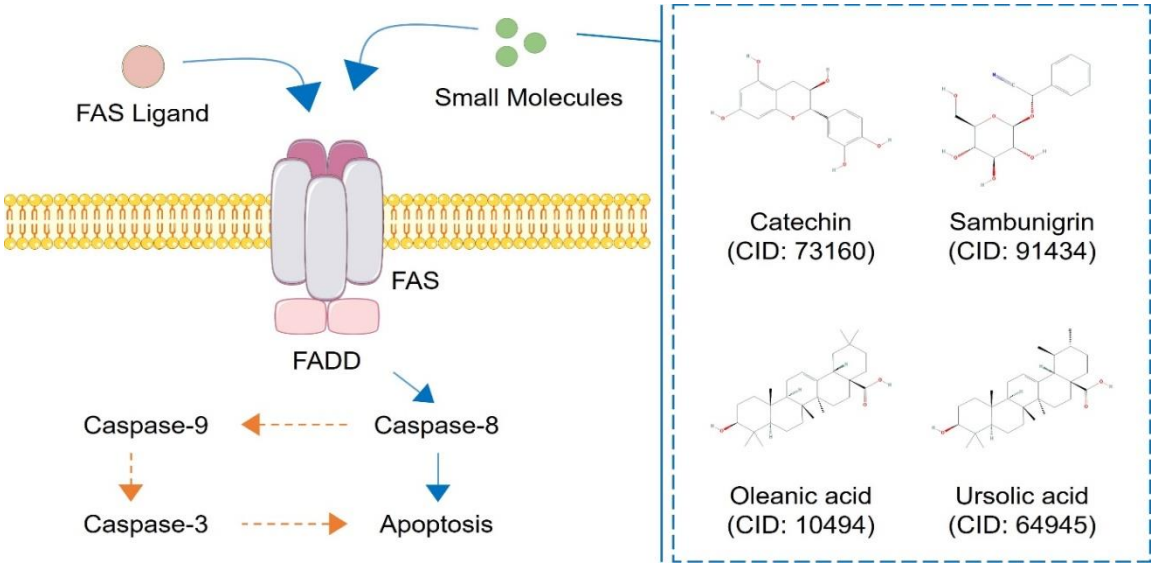

Fig. 1: The schematic illustration shows the possibility of extracellular death cell signaling pathways activation by small molecules including catechin, sambunigrin, oleanic acid, and ursolic acid via Fas

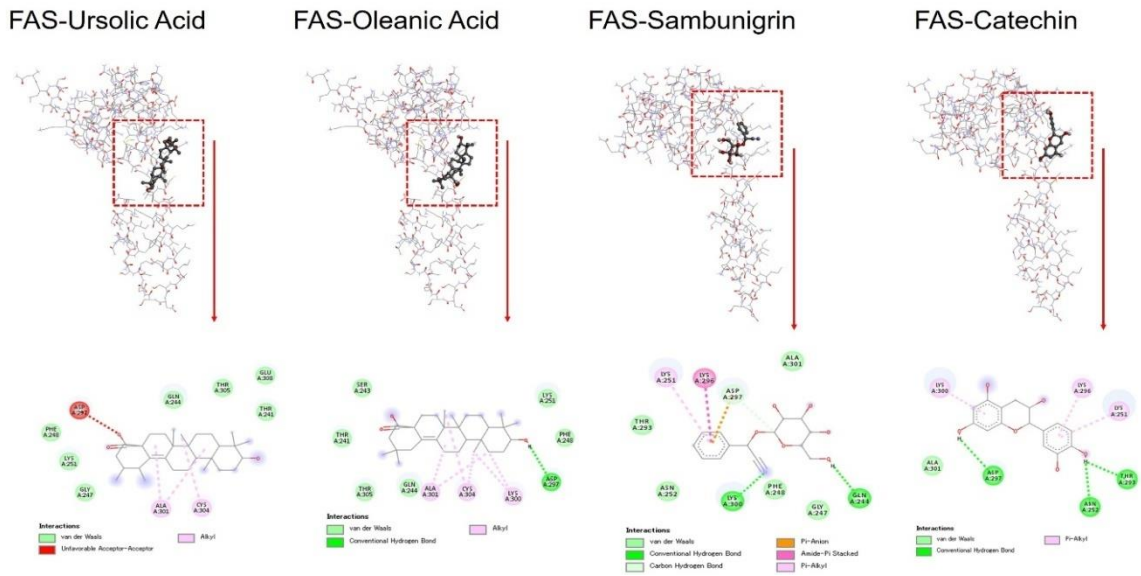

Fig. 2: Molecular interaction between the ligand and receptor. All bioactive compounds including ursolic acid, oleanic acid, sambunigrin, and catechin docked in proper area agonist Fas as a receptor

Table 2: Summarize of binding affinity and amino acid residues among receptor-ligand interactions

S/N	Molecular Interaction	Binding Affinity	Amino Acid Residues
1	FAS-Ursolic Acid	-6.6 kcal/mol	LYS251, ASP297, ALA301, THR241, CYS304, THR305, GLU308, PHE248, GLN244, GLY247
2	FAS-Oleanic Acid	-6.5 kcal/mol	LYS251, ASP297, ALA301, THR241, CYS304, THR305, LYS300, PHE248, GLN244, SER243
3	FAS-Sambunigrin	-6.2 kcal/mol	LYS251, ASP297, ALA301, ASN252, THR293, LYS296, LYS300, PHE248, GLN244, GLY247
4	FAS-Catechin	-6.1 kcal/mol	LYS251, ASP297, ALA301, ASN252, THR293, LYS296, LYS300

Molecular interaction between amino acid residues of Fas and the ligands defined the *Sambucus* bioactive compounds can bind with the Fas (Table 2). More specific, three amino acids including LYS251, ASP297, ALA301 were similarly found in the interaction with all compounds; ursolic acid, oleanic acid, sambunigrin, and catechin. Furthermore, the others residues such as THR241, CYS304, and THR305 interacted with ursolic acid and oleanic acid. On the other hand, the ASN252, THR293, and LYS296 also interacted with sambunigrin and catechin. Likewise, the GLY247 and GLY247 were connected to ursolic acid and sambunigrin. Besides, the PHE248 and GLN244 showed the interaction with ursolic acid, oleanic acid, and sambunigrin. And the last, LYS300 attached with oleanic acid, sambunigrin, and catechin. These results suggested that the similar amino acid residues that bind to small molecules have the same interaction probability (Asthana *et al.*, 2015).

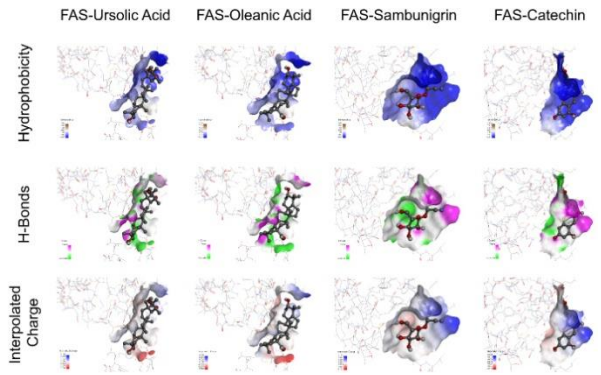

Fig. 3: Extended analysis of ligand and receptor interactions including hidrophobicity, hidrogen bonds, and interpolated charge features

The hydrophobicity, hydrogen bonds, and interpolated charge were shown as molecular interaction features between FAS as receptor and *Sambucus* bioactive compounds as the ligand (Fig. 3). It was also revealed that the binding site of Fas has negative potentials, which means that *Sambucus* bioactive compounds possess the positive potential that in turn facilitate them to bind with Fas. Electrostatic interaction has a pivotal role in protein-ligand interaction for complex formation. The hydrophobicity, hydrogen bonds, and interpolated charge characters are essential features to justify the stability of protein-ligand interaction (Patil *et al.*, 2010).

Conclusion

Based on the *in silico* study, it was shown that *Sambucus* bioactive compounds including ursolic acid, oleanic acid, sambunigrin, and catechin have a high possibility to interact with Fas with following binding affinity -6.6, -6.5, -6.2, and -6.1 kcal/mol, respectively. Furthermore, it was shown that the amino acid residues that bind with ligands, namely Fas-ursolic acid (LYS251, ASP297, ALA301, THR241, CYS304, THR305, GLU308, PHE248, GLN244, GLY247), Fas-oleanic acid (LYS251, ASP297, ALA301, THR241, CYS304, THR305, LYS300, PHE248, GLN244, SER243), Fas-sambunigrin (LYS251, ASP297, ALA301, ASN252, THR293, LYS296, LYS300, PHE248, GLN244, GLY247), and Fas-catechin (LYS251, ASP297, ALA301, ASN252, THR293, LYS296, LYS300). Finally, the hydrophobicity, hydrogen bonds, and interpolated charge governed the binding affinities among the ligands and the Fas as a targeted receptor. Therefore, it suggests that the *Sambucus* bioactive compounds might have pro-apoptotic activity through inducing death cell extrinsic signal pathway via Fas.

Conflict of Interest

The author declares there is no conflict of interest in this work.

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