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α-solanine and α-chaconine from Potato (*Solanum tuberosum*) for Endometrial Cancer Therapy: *In silico* Study

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Abstract: The study aims to identify the potency of α-solanine and α-chaconine in endometrium cancer. Whether it could increase the cancer risk or be an anti-cancer agent. The α-solanine (CID9549171), α-chaconine (CID442971), and cordycepin (CID452851) structures were generated from MolView by inputting the SMILES from PubChem of each compound. The 3D structure of adenosine (CID60961) was downloaded from PubChem. Adenosine A2 Receptor (A2AR) (2YDO) as a macromolecule was downloaded from PDB. Protein and ligands were docked using Molegro Virtual Docker 5. The docking results were analyzed using Pymol and Discovery studio 21.1.1 and visualized in 2D and 3D visualization. The α-solanine and α-chaconine were predicted for their pharesiamacological effect using PASS Online. The result showed that α-solanine and α-chaconine were able to bind to A2AR active sites. The amino acid residues involved in the α-solanine and α-chaconine binding with A2AR were identical. Interestingly, the result showed that α-solanine can behave like cordycepin when it interacts with A2AR. The binding site of cordycepin – A2AR is most likely similar to α-solanine – A2AR complex. It could be assumed that α-solanine has similar potency with cordycepin as anti-cancer. In addition, the pharmaceutical prediction of α-solanine and α-chaconine showed the potency as anti-cancer for both compounds. This study predicted that α-solanine and α-chaconine, which are known as toxic, could be anti-cancer agents. The α-solanine could work in a similar mechanism as cordycepin, whereas the α-chaconine mechanism as anti-cancer needs further identification.

Keywords: A_2AR, cordycepin, docking, endometrial cancer, α-solanine, α-chaconine.

Abbreviations: GA=Glycoalkaloid, EC=Endometrial cancer

Introduction

Glycoalkaloids (GAs) are nitrogen-containing steroidal glycosides that are generated from potatoes (*Solanum tuberosum*) and all nightshade plants. GA is a natural pesticide that functions to protect plants against environmental attacks from insects, bacteria, and animals. The major GA compounds in potatoes are α-solanine and α-chaconine (Sotelo et al., 2000; Friedman, 2006). Those GAs are green-colored and visible to be seen by eyes if the concentration is high (Omayio et al., 2016). The α-solanine and α-chaconine are beneficial for plants and they won't harm if consumed in low concentration. However, it causes some cases of poisoning ranging from mild symptoms to severe symptoms, such as nausea, dizziness, abdominal pain, diarrhea, convulsions, coma, and death (Langkilde et al., 2008; Chen et al., 2021).

The poisoning cases, such as changes in breathing and coma, are related to the inhibition of cholinesterase activity in the central nervous system. Previous in vitro and in vivo studies found

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the inhibition of AChE and BuChE activity and disruption of the cell membrane in the gastrointestinal tract by α-solanine and α-chaconine (McGehee et al., 2000; Langkilde et al., 2008; Schrenk et al., 2020). Aside from the effect on the central nervous system and cell membrane, α-solanine and α-chaconine also cause risk in the reproductive system (Chen et al., 2021). A recent animal study showed the inhibition of mouse sertoli and Leydig cells by α-solanine and α-chaconine (Park et al., 2019). High dose consumption could induce abortion in pregnant mice (Friedman et al., 2003) and cause lethal in hamsters (Langkilde et al., 2008). It is also toxic to oocyte maturation then suppresses the embryonic development in the pig model (Lin et al., 2018). Contrary to the findings of α-solanine and α-chaconine toxicity, other findings showed the potency of α-solanine and α-chaconine for cancer treatment and therapy via cell cycle protein expression, activation of P38 MAPK pathway (Pan et al., 2016), and mitochondria-mediated cell apoptosis (Sun et al., 2014).

Adenosine receptors belong to a family of G protein-coupled receptors (GPCRs), which have four types (A_1R, A_2AR, A_2BR, and A_3R) and are activated by extracellular adenosine. Among all those types of adenosine receptors, A_2AR is mostly chosen to be the target protein for cancer treatment and therapy (Congreve et al., 2018). It is expressed in various immune cells and endothelial cells (Vigano et al., 2019). Ohta et al. (2006) defined the importance of A2AR in regulating**THE** immune response of adenosine-regulated effector T cells. Due to its distribution and dynamic expression pattern on immune cells, A_2AR could be the potential target for cancer treatment and therapy. But here, we focused our research of A_2AR on endometrial cancer (EC) mechanism as it keeps increasing every year. According to the GLOBOCAN cancer statistics, there are approximately 382,069 new cases and 89,929 deaths caused by EC in 2018 (Bray et al., 2018). The incidence rates of EC were found higher in high-income countries than in low to middle-income countries. Contrarily, the mortality case of EC was the highest among women with low socioeconomic status (Zhang et al., 2019).

There was numerous research identified about the potency of natural bioactive compounds to inhibit EC cancer. Huang et al. (2021) described the potency of Coix or adlay (*Coix lachryma-jobi* L. var. Ma-yuen Stapf.) as anti-cancer via cell growth inhibition. Cordycepin from *Cordyceps sinensis* and *Cordyceps militaris* known for its ability to reduce the risk of EC. Cordycepin inhibits tumor growth by tumor cell apoptosis and inhibits the tumor metastasis mechanism. Besides that, cordycepin is also known for its potency in clinical health including immunomodulatory, antioxidant, anti-inflammatory, and anti-microbial activities.

Considering the emergence of EC incidence and the gap of α-solanine and α-chaconine toxin in cancer treatment and therapy, this study aims to predict the potency of α-solanine and α-chaconine as EC therapy. Whether it could be beneficial or unsafe to be consumed. This study used cordycepin for comparison, as cordycepin had been known for its potency as an A2AR agonist that is beneficial in EC cancer treatment.

Materials and Methods

Protein and Ligand Retrieval

The 3D structure of the A2AR (2YDO) protein was downloaded from PDB. The 3D structure of α-solanine and α-chaconine were generated by inputting the SMILES of α-solanine (CID9549171) and α-chaconine (CID442971) from PubChem. The 3D structure of cordycepin (CID452851) was downloaded from PubChem.

Docking

Docking was done with Molegro Virtual Docker 5. The A2AR were docked to ligands in the specific grid X -30,3; Y 5,81; Z -19,84 with docking parameter MolDock Grid 0,5; RMSD < 2, and 10x repetition. The docking results were analyzed using Pymol and Discovery studio 21.1.1. Interaction of protein and ligands were visualized in the 3D and 2D. The binding energy of ligands to protein will be analyzed with the following formulation ΔG = Moldock Score + Moldock Grid Score + Rerank score.

Biological Activity Prediction

SMILES of α-solanine and α-chaconine were used to predict the biological activity in PASS Online (http://way2drug.com/passonline). The analysis was done by identifying Pa (activity) of the compounds that are related to cancer and immune response.

Results and Discussion

α-solanine and α-chaconine binding to A_2AR

The binding of the native ligand of A_2AR, adenosine, to A_2AR was used to identify the active sites of A_2AR. The following amino acid residues involved in the adenosine - A_2AR (Table 1; Figure 1a) binding are considered as the active sites of A_2AR, Asn253, His278, Thr88, Leu249, Phe168, Ile274, and Met177. By the docking result, similar amino acid residues exist in both α-solanine and α-chaconine binding to A_2AR (Table 1; Figure 1b-c). The α-solanine built interaction with the A_2AR with these following amino acid residues, Lys150, Asp170, Glu169, Ala265, His264, Ala63, Leu167, Leu249, Met270, Ile274, Met174, Leu267, Val84, Phe168, His264, Tyr271 and the binding was formed by hydrogen bond and hydrophobic interaction. The α-chaconine formed hydrogen bond and hydrophobic interaction with A2AR through Lys153, Asp170, Ser67, Ala265, Ala63, Ile66, Val84, Ile274, Leu249, Phe168, Trp246, Tyr271, and His278.

From the amino acid residues involved in the α-solanine and α-chaconine binding to A_2AR, some amino acid residues were found in both interactions, there were Ala63, Val84, Phe168, Asp170, Leu249, Ala265, Tyr271, and Ile274. Where, three of them, Phe168, Leu249, and Ile274, were the amino acid residues that constitute active sites of A_2AR (Dal Ben et al., 2019). It indicates the ability of α-solanine and α-chaconine to perform their potency to A_2AR, whether as an agonist or antagonist. The similarity of α-solanine and α-chaconine binding sites to A_2AR, presumably because of the similar structure.

Besides the amino acid residues in the complex, binding affinity also affects the optimum binding of protein and ligand. According to the calculation of ΔG, the strongest binding was constituted by α-chaconine. Surprisingly, the α-chaconine - A_2AR binding was much stronger than A_2AR binding to the native ligand. Conversely, the α-solanine binding to A2AR has a likely similar binding affinity score with adenosine - A_2AR. It indicates the ability of α-solanine to behave as adenosine more than α-chaconine.

α-solanine and α-chaconine for endometrial cancer therapy

Cordycepin performed binding with A_2AR (Table 1; Figure 1d) through Lys150, Phe168, Tyr271, Asp170, Asn253, Asn181, His278, Tyr271, Glu169, His264, Leu249, Leu267, Met177, Leu167, Leu249, Met270, Ile274, and Leu85. Interestingly, α-solanine posed binding with the similar amino acid residues as cordycepin - A_2AR complex. The result is contradictory to the previous study.

The similar amino acid residues that occurred in α-solanine - A_2AR complex and cordycepin - A_2AR complex were Lys150, Asp170, Glu169, His264, Leu167, Leu249, Met270, Ile274, Leu267, Phe168, His264, and Tyr271 with hydrogen bonds and hydrophobic interaction. In addition, α-chaconine also binds to A_2AR with similar amino acid residues as cordycepin to A_2AR. However, the number of amino acid residues involved in the α-chaconine - A_2AR binding was not as much as α-solanine A_2AR binding. This study assumed that α-solanine could perform as an A_2AR stimulant with the same mechanism as cordycepin.

Table 1. Interaction of α-solanine, α-chaconine, and cordycepin, and adenosine with A2AR

Protein	Ligand	Name	Category	Energy (kJ/mol)
A2AR	α-solanine	**Lys150**, **Asp170**, **Glu169**, Ala265, **His264** Ala63, **Leu167**, **Leu249**, **Met270**, **Ile274**, Met174, **Leu267**, Val84, **Phe168**, **His264**, **Tyr271**	Hydrogen bond Hydrophobic	-1413.41
	α-chaconine	Lys153, **Asp170**, Ser67, Ala265 Ala63, Ile66, Val84, **Leu249**, **Phe168**, Trp246, **Tyr271**, **Ile274**, His278	Hydrogen bond Hydrophobic	-2399.23
	Cordycepin	Lys150, Phe168, Tyr271, Asp170, Asn253, Asn181, His278, Tyr271, Glu169, His264 Glu169 Leu249, Leu267, Met177, Leu167, Leu249, Met270, Ile274, Leu85	Hydrogen bonds Electrostatic Hydrophobic	-1882.09
	Adenosine (C)	Asn253, His278, Thr88 Leu249, Phe168, Ile274, Met177	Hydrogen bond Hydrophobic	-1368.19

Note: **Bold is the amino acid residues in Cordycepin – A2AR,** Red is the amino acid residues inactive sites (Adenosine – A2AR)

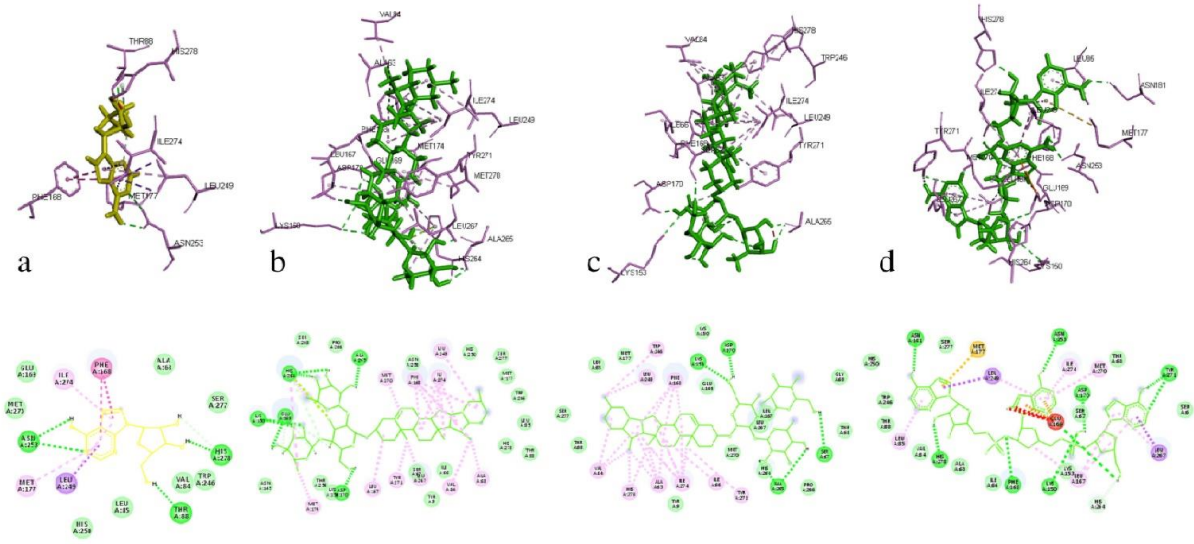

Figure 1. 3D and 2D visualization of (a) adenosine, (b) α-solanine, (c) α-chaconine, and (d) ccordycepin binding to A2AR

Discussion

Extracellular adenosine performed activation in cellular signaling pathways through G-protein-coupled receptors. The A2a receptor is a high-affinity receptor and is expressed on T cells and natural killer T cells, monocytes, macrophages, DCs, and natural killer (NK) cells. The A2ARis upregulated in macrophages in response to NF-κB, STAT1, and PPAR**γ** and adenosine signaling. The A2AR activation suppresses the secretion of neutrophils chemoattractant, thus blunting the inflammatory response (Leone & Emens, 2018). Nowadays, A2AR is being the target of an anti-cancer mechanism. The agonist mechanism of ligands will lead to anti-inflammatory mechanisms through A2AR stimulation and it is possible to reduce the risk of cancer.

From the result, this study could predict the ability of α-solanine and α-chaconine to construct binding with A2AR. However, the mechanism of α-solanine and α-chaconin2AR still remains unknown. To identify the potency of α-solanine

and α-chaconine as anti-cancer via A2AR, a known bioactive compound for cancer treatment was used as a comparison. Cordycepin from *Cordyceps sinensis* has exhibited some beneficial effects including immunomodulatory, anticancer, antioxidant, anti-inflammatory, and anti-microbial activities (Jin et al., 2018; Özenver et al., 2021). This compound is a derivative of adenosine, which may have an agonist mechanism to A2AR. It could stimulate A2AR to perform an anti-inflammatory activity (Pan et al., 2016; Du et al., 2021). Fong et al. (2018) reported that cordycepin has potency in inhibiting EC cells by inducing cellular apoptosis in Ishikawa cells. Further investigation found that cordycepin has the potential to interfere with the expression of mRNA and affect DNA synthesis. Thus, the cell cannot proliferate and enter the synthesis phase, resulting in apoptosis and cell death.

The potency of α-solanine as an anti-cancer agent in endometriuthe m with cordycepin-alike mechanisms is the novelty of this study. Thus, α-solanine is not only toxic but also hurts the female reproductive system (Chen et al., 2021). Aside from the negative effect on the female reproductive system, a previous study also mentioned the toxicity of α-solanine and α-chaconine, It stated that α-solanine and α-chaconine in some concentrations (3-6 mg/kg of body weight) can cause toxic symptoms such as cell membranes disruption, teratogen effect, and cholines inhibition (Kuete, V. (2014; Romanucci et al., 2016). In contrast, α-solanine showed the potency to reduce the risk of endometrial cancer. A study by Hassan et al. (2019) mentioned that α-solanine showed cytotoxicity against various cancer cell lines through several mechanisms. Applying α-solanine to the endometrium (observed in RL95-2 cell lines) would be able to downregulate the expression of p-Akt and p-Erα. Additionally, this study found that α-solanine may perform the anti-cancer mechanism for endometrium via A2AR agonist.

As for α-chaconine which still remainown for its potency, this study predicts the bioactivity of α-chaconine through PASS online (data not shown). It showed that α-chaconine had some anti-cancer properties. The α-chaconine has immunosuppressant properties, is anti-carcinogenic, apoptosis agonist, anti-inflammatory, and could be adenosine regulator but the activity might below. The result, it could explain the mechanism of α-chaconine as an anti-cancer. The A2AR agonist performed an anti-cancer mechanism via apoptosis and anti-inflammatory (Fong et al., 2018; Leone & Emens, 2018). Thus, the anti-cancer mechanism of α-chaconine might be similar to α-solanine, but α-chaconine is performed with a different receptor.

Conclusions

In conclusion, the α-solanine has potency as endometrial cancer therapy via A2AR agonist, as same as cordycepin. The α-chaconine predicted has potency as anti-cancer too, however, the mechanism remains unclear. Further study is required to identify the mechanism of α-chaconine as an anti-cancer.

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Conflict of Interest: The authors declare that there are no conflicts of interest concerning the publication of this article.

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