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Tomatidine Extraction from Various Solvents and Cancer Inhibition Mechanism Through *In Silico* Analysis

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Abstract

Tomatidine is an alkaloid-steroid contained in tomato plant organs that potentially developed as an anti-cancer molecule. However, inappropriate use of solvents is frequently becoming an obstacle in the extractions process. In addition, the mechanism of action of tomatidine in the inhibition of cancer cell signaling pathways needs to be investigated. Therefore, this research aims to discover an appropriate solvent to extract tomatidine effectively, and to analyze the inhibition mechanism of tomatidine against the cancer growth pathway. A total of 80 kg of fresh tomatoes were cut into pieces and steamed at 120 °C for 30 min, then mashed and soaked for 3 days in 70 % ethanol, aquadest, petroleum ether, and chloroform separately, continued with GC/MS analysis. Molecular docking was conducted using targeted proteins including B-Raf proto-oncogen serine/ threonine protein kinase (BRAF), Vascular endothelial growth factor A (VEGFA), Smoothened protein (SMO), Cyclin-dependent kinase 2 (CDK2), and Tyrosine-protein kinase/ Janus kinase-1 (JAK1). The ligand-receptor was performed using AutoDock Vina v1.2.0. The highest extracted tomatidine was found in chloroform at 2.44 % of total alkaloid-steroid, and cannot be found in water. The highest interaction is observed in the SMO-tomatidine interaction complex, followed by JAK-1. Furthermore, physicochemical properties of tomatidine make it invaginated into the hydrophobic side of the target proteins that are mainly triggered by van der Waals and alkyl interaction. Based on the in-silico study, tomatidine significantly interacted in the oncogenic signaling pathways that therapeutically potential inhibit cancer cell proliferation. Further study should be focused on the effectiveness analysis of the SHH signaling pathway in the cancer cell.

Keywords: Alkaloid-steroid, Non-polar solvent, Phytosterol, SHH cascade, Therapeutic

Introduction

Tomato (*Lycopersicum esculentum* Linn.) contains abundant secondary metabolites, such as tomatidine. It is an alkaloid-steroid that can be extracted from the fruit skin and leaves. Tomatidine is the aglycon metabolite of tomatine shows exert various beneficial biological activities such as anti-cancer, anti-inflammatory, and muscle health promotion molecules. Tomatidine potentially reduces the replication of the pathogenic bacterium *Streptococcus aureus* [1], and has an antiviral activity against several types of viruses including Sunn-Hemp Rossette virus, human herpes simplex virus, human respiratory syncytial, and influenza virus [2].

The development of metabolites as therapeutic compounds is frequently hampered by an appropriate extraction method. Hence, extraction analysis is needed to determine how the extraction process should be conducted especially based on the solvent types. It is because, the extraction process is strongly influenced by the solvent polarity [3], and they have shown different results in the process of extraction by secondary metabolism [4]. Specifically, tomatidine is potentially developed as a drug candidate for treating cancer

cells [5,6]. *In vitro* studies have shown the potential of tomatidine in suppressing the proliferation and growth of human gastric cancer-derived 85As2 cells through inhibition of the interferon signaling pathway [7]. Furthermore, tomatidine is also known to be able to inhibit the invasion of the HT1080 fibrosarcoma cell line through p38 and p38 inactivation extracellular signal-regulated pathway (ERKs) [8], and inhibits osteosarcoma HOS and U2OS cell lines through c-Raf-MEK-ERK and presenilin 1 pathway [9]. The mechanism of inhibition of tomatidine against cancer growth is very likely to be different and involve other signaling proteins depending on the type of cancer. Hence, further studies are needed as a fundamental baseline for developing effective medications.

An effective analysis to depict the tomatidine therapeutic potential action is able to the method of docking simulation. It helps in explaining how tomatidine roles on molecular signaling involved in cancer pathophysiology cascade. Therefore, the in-silico approach was chosen as a stepstone in determining target proteins for inhibition of cancer growth pathways. These results can then be used in considering target proteins and treatment models that should be developed. Therefore, this research aims to discover an appropriate solvent to extract tomatidine effectively, and to analyze the inhibition mechanism of tomatidine against cancer growth pathways.

Materials and methods

This study was an observational explanatory to determine the most effective solvent, and qualitatively identify the inhibition mechanism of tomatidine against cancer growth. The research was conducted at the Nutrition Laboratory, Faculty of Nutrition Science, Universitas Katolik Soegijopranoto (UNIKA) for the extraction process. The quantification of tomato bioactive compounds was carried out at the Integrated Research and Testing Laboratory (LPPT), Universitas Gadjah Mada (UGM), Yogyakarta, Indonesia.

Tomato extraction

A total of 80 kg of fresh tomatoes of the Lentana variety were obtained from the Bandungan vegetable center, Semarang Regency, Central Java, Indonesia. Tomatoes that have been washed using clean water and removed the calyces, were cut into small pieces and steamed at 120 °C for 30 min. The steamed tomatoes were then mashed and divided into 4 parts which were placed in a plastic container with a capacity of 5 L. The extraction process was carried out by adding 70 % ethanol, aquadest, petroleum ether, and chloroform separately in each plastic container to a volume of up to twice as much as steamed tomatoes. The tomato and solvent mixture were then allowed to stand for 24 h, then the tomato solution was evaporated to obtain a paste for gas chromatography/mass spectrophotometry (GC/MS) analysis.

GC/MS analysis

A total of 0.3 mL of each sample was redissolved using 1 mL of MeOH in Eppendorf and vortexed until homogeneous and diluted by centrifugation for 3 min at a speed of 9,000 rpm. The supernatant was then transferred into a GC vial to be injected into the GC/MS machine. The machine used is the Thermo Scientific TraceTM 1310 Gas Chromatography (Thermo Fisher Scientific: Waltham, USA). The instrument specifications are Column: HP-5MS UI, the carrier gas used is Helium UHP (He) with an injector temperature of 260 °C. The molecular separation rate is 50 mL/min with a ratio of 50:50 and front inlet flow: 1.00 mL/min. The temperature of the MS-transfer line is 250 °C and the ion source is 200 °C, the purge flow is 3 mL/min, the gas saver flow is 5 mL/min and the gas saver time is 5 min.

Protein receptor determination

The target signaling pathway was initiated by downloading the molecular formula of tomatidine based on the canonical Simplified Molecular-Input Line-Entry System (SMILES) from PubMed. (https://pubchem.ncbi.nlm.nih.gov). The query for the tomatidine molecule was then inputted on the SwissTargetPrediction online platform (http://www.swisstargetprediction.ch), and it generates approximately 100 predictions of pathways that have the potential to interact with tomatidine. The signaling pathway obtained was then reduced and confirmed through the pathway in cancer based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.kegg.jp/pathway/map05200). Based on these stages, 5 target proteins were obtained from various cascades involved in the evading stages of apoptosis, proliferation, and angiogenesis (**Table 1**).

Table 1 Information on oncogenic target proteins and signaling pathways involved in cancer.

Protein name	Interacted proteins	Pathway	Role in cancer
B-Raf proto-oncogen serine/ threonine protein kinase (BRAF)	KRAS2, MAPK1, ERK,	MAPK signaling pathway	Evading apoptosis, proliferation, angiogenesis
Vascular endothelial growth factor A (VEGFA)	ERK, EGFR, GLUT1	HIF-1 and VEGF signaling pathway	Survival and angiogenesis
Cyclin-dependent kinase 2 (CDK2)	P53, CCND1, RB1,	Cell cycling	Proliferation
Smoothened protein (SMO)	PTCH1, SUFU	Sonic Hedgehog signaling pathway	Proliferation
Tyrosine-protein kinase/ Janus kinase-1 (JAK1)	IFN, STAT1, PI3K	JAK/STAT signaling pathway	Evading apoptosis, proliferation

The selection of these proteins as representatives of the main signaling pathways in the metastatic stage of cancer cells. Determination of the linkage of the 5 protein targets was carried out through the *in silico* simulating pathway using STRING software (https://string-db.org/) to identify related proteins.

Preparation of ligands and receptor proteins

The 3D structure of the tomatidine compound downloaded from PubChem was used as a ligand, then prepared using the OpenBabel 2.4.0 program [10], in the PyRx 0.8 application to minimize and get the ligand file in *pdbqt format. Then, the 3D structures of the 5 target proteins were downloaded from the RCSB (https://www.rcsb.org/) and prepared using the VEGAZZ ver. application. 3.2.2.21 set up for Windows powered by CMimple Org [11]. Protein preparation was carried out for the removal of water molecules, natural ligands, and unneeded residues, as well as the addition of hydrogen molecules.

Molecular docking and visualization

Molecular docking was performed to anchor the ligand to the prepared receptor protein. The predicted binding energy was between the binding of tomatidine with proteins SMO (ID: 4JKV), JAK1 (ID: 4E4L), and VEGFA (ID: 1BJ1), BRAF (ID: 3NY5), and CDK2 (ID: 1B39). The type of molecular docking used was blind docking where the grid box parameters of the proteins were unknown before, and carried out using AutoDock Vina v1.2.0 [12,13], integrated PyRx 0.8. Protein molecules were uploaded and added as macromolecules in *pdbqt format, then ligands that were prepared previously using Open Babel were added. Next set the grid box on the receptor protein. The results of docking between ligands and receptor proteins were indicated by the value of binding affinity and visualized using the BIOVIA Discovery Studio Visualizer 16.1 application [14,15]. Visualization results were exported in 2D and 3D forms to determine the amino acid residues and the bonds formed. The validation docking results was confirmed when the root mean square deviation (RMSD) value is less than 2 Å, which indicates there was no fluctuation in the amino acid and ligand interaction [16].

Results and discussion

The extraction procedure is a crucial step in obtaining the target compound. An understanding of the physicochemical character of the target compound becomes very important as a basis for thinking in determining the type of solvent to be used [4]. In this study, the extraction of tomatidine which is a non-polar compound belonging to the phytosteroid group is more suitable to be carried out using chloroform, petroleum ether, or ethanol with a purity of 70 % or more. Generally, the atomic interactions greatly affect the physicochemical characteristics of the bioactive compounds, such as pH, polarity [17], and thermal stability in the solvent [18]. Tomatidine content is shown below 2.50 % after extraction of the green raw tomatoes in different solvents. Indeed, tomatidine is not detected in a mixture of high polar solvent and aquadest. High tomatidine content was found in chloroform, followed by petroleum ether and ethanol (**Table 1**).

Table 1 Extraction composition of tomatidine from tomato fruit in various solvents.

IUPAC name	Molecule formula	PubChem CID	Mol. weight ($g.mol^{-1}$)	Composition (%)			
				P	C	E	A
Tomatidine	$C_{27}H_{45}NO_2$	65576	415.662	1.05	2.44	0.09	-

Note: P = petroleum ether; C = chloroform; E = 70 % ethanol; A = Aquadest.

Show in **Table 1**, the extraction composition (%) are shown differently according to solvent polarity, then the extraction composition of tomatidine is shown highest in the chloroform. It may because the chloroform and petroleum ether have low dielectric constants and dissolve non-polar compounds with the same internal pressure through induced dipole interactions [19]. Material solubility in low polarity solvents is determined by the weak momentary dipole force in the Van-Der-Waals interaction. Then, the solubility rate in a polar solvent is determined by the ionization process [20]. Meanwhile, in 70 % ethanol, the instantaneous dipole force can affect polarity degree which causes non-polar compounds to dissolve [21].

In general, phytosterol compounds have a tetracyclic structure, with an additional side chain at the C-4 and C-24 sites [22]. The unsaturation properties of the phytosterol are influenced by the presence of side chains and tetracyclic rings. Furthermore, the conjugation of an alcoholic hydroxyl group to the C-3 site creates charged molecule faction and potentially binds to phenolic acids, fatty acids, or carbohydrates [23]. In addition, several free phytosterols contain a double bond in the B-ring between C-5 and C-6 referred to Δ5-phytosterol, or C-5 and C-6 called Δ7-phytosterols which is usually used as an identifier/ distinctive character of certain plant species [22]. For example, phytosterols in the majority of plants have a Δ5-phytosterol structure, but certain species from the Amaranthaceae Juss. and Cucurbitaceae Juss. families are dominated by the Δ7-phytosterol structure [24]. However, the B-ring's double bond structure seems disappear in tomatidine and replaced with hydrogen atoms in C5 and C8 (**Figure 1**).

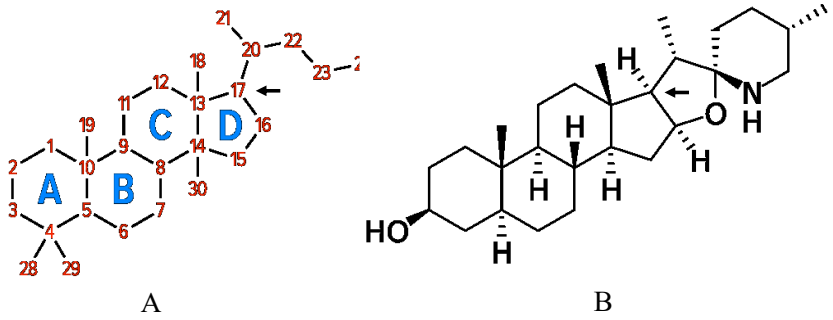

Figure 1 Comparison of the chemical formulas of steroids (A) and tomatidine (B) contained in tomatoes as ligands. The arrows indicate the location of the side chain branches.

Phytosterols can be grouped using the presence of a methyl at the C-4 atom, which becomes desmethyl- (without metal groups), 4-monomethyl- (1 methyl molecule linked to C-4), or 4,4-dimethyl sterol (2 methyls). In addition, the presence of an alkyl group at the C-24 atom is also used as a basis for classifying the structural diversity of phytosterols in higher plants where methyl, methylene, ethyl, or ethylene groups can be attached to C-24 [25]. Furthermore, some conditions that are quite rarely found in phytosterols are the presence of double bonds in the C-22 and C-23 side chains or C-23 with C-24 which increases the polarity of the sterols. The limited polar molecular group (only at C-3 and possibly at the free end) causes phytosterol compounds to be insoluble in non-polar compounds [26].

Based on the information above, the comparison of tomatidine chemical structure with phytosterols generally shows differences, such as the free C-4 end and C-3 containing hydroxy groups in tomatidine. In addition, the branching at C-23 binds to N-24 and leaves an end that may result in a change in solubility polarity. The physicochemical properties of phytosterols, tomatidine does not provide enough polar site (OH^-) to attach water molecules. Therefore, extraction concentration of tomatidine is shown very low as 0.009 in 70 % ethanol. Since the polarity of aquadest is lower comparatively than petroleum ether and chloroform, the extraction ability of tomatidine is shown lower in aquadest. Tomatidine is a phytosteroid that can naturally be found in green tomatoes and leaves and degrades in ripe tomatoes [27]. Therefore, the

tomato age selection factor may also be the cause of the low concentration of tomatidine. Further studies are needed to find the best concentration of tomatidine in various types and ages of tomatoes.

Molecular docking

The chemical structure of phytosterols, such as tomatidine, are potentially to be developed as superior molecules to inhibit various cascades related to cancer cell activity. In general, steroid compounds can directly insert cell membranes and enter cancer cells and activate signaling pathway, directly [26]. Then, this phytosterol characteristic becomes theoretical basis for simulating the signaling interactions between tomatidine as a ligand and several oncogenic proteins.

The results of molecular docking between the tomatidine ligand and 5 targeted protein receptors are performed by the binding affinity values which indicate the compound's ability to interact with (**Table 2**). The negative results indicate the suitability of the efficient ligand-receptor site binding simulation with low-affinity energy requirements. Therefore, the lower affinity value produced by the ligand-receptor interaction is predicted as the best binding model in the docking simulation [28].

Table 2 Value of binding affinity from molecular docking.

Protein receptor	Binding affinity (kcal/mol)	RMSD (Å)	Amino acid is involved in the direct interaction
CDK2	–8.3	0.830	Leu134(A)[b], Ile010(A)[b], Val064(A)[b], Ala031(A)[b], Ala144 (A)[b], Val018 (A)[b], Glu012(A)[d]
BRAF	–8.2	1.041	Pro162 (A)[a], Phe160(A)[e], His223(A)[c], Val226(A)[b].
VEGFA	–7.4	1.745	Phe132(A)[e], Phe118(L)[e], Val133(L)[b], Leu151(H)[b], Leu135(L)[b]. Val153(L)[b], Val191(L)[b]
JAK1	–10.0	1.412	Trp1047(A)[e]. Pro1044(A)[b], His885(A)[c], Leu1024(A)[b]
SMO	–11.0	1.050	Ala459(A)[b], Phe332(R)[e], Leu412(R)[b], Val329(R)[b], Ile408(R)[b], Met525(R)[b], Ile413(R)[b], Leu405(R)[b]

Note: RMSD value ≤ 2 Å indicates there are no fluctuation in the amino acid bond with the ligand during the docking process. superscript alphabet indicates side chain (R group) amino acids charges: [a)] polar, uncharged; [b)] non-polar, aliphatic; [c)] positive charge; [d)] negative charge; [e)] aromatic.

The validation results performed by the RMSD values show that the ligand-receptor bond is less than 2 Å, so it is feasible to describe the interaction between ligands and amino acid side residue chains. Furthermore, ligand-receptor interactions in all target proteins are dominated by non-polar aliphatic R group amino acids, which are hydrophobic that make them folded inward. This is also probably triggered by the structural characteristics of the steroid which is dominated by covalent bonds and less polar bonds. These interactions are then depicted in 2D and 3D imaging models of ligand and receptor docking results.

Docking visualization

An interesting finding from the simulated interaction suggests that the tomatidine binding site resides within the hydrophobic groups of all target proteins, mainly formed by α-helix structure (in CDK2, JAK1, and SMO proteins) and β-sheet structure (in BRAF and VEGFA proteins) (**Figure 2**). Furthermore, the docking simulation in this study shows that the interaction of Van der Waals and alkyl is the most dominant in all docking models. This indicates that the interaction of tomatidine with the target protein is caused by instantaneously induced polishing and weak non-covalent interactions [29], due to the fluctuating polarization of nearby particles. The alkyl interactions also allow the opening and folding of the 2 alkyl molecular chains thereby allowing the immersion of the hydrophobic fraction into the protein molecule [30]. Therefore, the interaction of tomatidine with various target proteins is on the hydrophobic side of the protein.

Specifically, the interaction formed between the tomatidine compound and CDK2 protein is between β-sheet structure near the C-terminal end, and α-helix near the N-terminal end. Ligand-receptor interactions are mainly formed from residues of 4 main amino acids, namely alanine (Ala), valine (Val), leucine (Leu), and isoleucine (Ile) which are bound to the tetracyclic structure of the A-C ring. The ligand-receptor complex formed Pi-alkyl interactions at residues Ile-10, Val-18, Ala-31, Val-64, Leu-134, and Ala-144. In addition, an interesting finding in this simulation shows that there is a hydrogen bond between 1 glutamate

(Glu-12) and N-23 tomatidine (**Figure 3(A)**). This supports the previous research that side chain branching allows the formation of ionic bonds which may also play a role in the solubility of tomatidine in polar solvents [31].

In contrast to the CDK1 protein, where the Van der Waals and alky interactions by ligands with residues Pro, Val, Glu, His, Leu, Lys, and Asn, the interaction of tomatidine with BRAF protein mainly performs pi-alkyl interactions (**Figure (3B)**). This interaction indicates the possibility of a bond between benzene group of the protein residues and alkyl ligand group from tomatidine, and vice versa [32]. The electron affinity induced by the ligand allows non-covalent interactions with electrons in the benzene ring of protein residues [33], where there are only 3 amino acids with the benzene structure. This is relevant to the presence of phenylalanine (Phe-160) which is recruited and interacts with the tomatidine side chain at the C-24' atom to form a Pi-alkyl bond.

Interesting findings were found in the interaction between tomatidine and VEGFA protein which involves about 26 amino acids and 6 types of interactions. Where 4 residues including Leu-134, Val-191, His-174, and Phe-176, form an unfavorable donor-donor interaction that represents a mismatch in the binding position between the ligand and the receptor. This is probably happen caused by no limitation in the flexibility calculations of the docking protocol in this study. It makes the interaction of the ligand with the receptor occurs rigidly and based on the suitability of the binding site that majority formed by the protein residues. Furthermore, the computational induced-fit effect will automatically take into account the fit and match of the ligand to the simulated protein [34].

Unfavorable donors also indicate the possibility of an unactual target protein binding site from the simulation results. In other words, unfavorable donor-donor interactions may be kinetically unfavorable because it increases the high energy cost of rotating the bonding cavity thereby increasing the risk of developing unintegrated parts or unbinding sites [35]. Therefore, the interaction between tomatidine and VEGFA has the lowest interaction, below −8 kcal/mol. The presence of phenylalanine was also observed in the formation of pi-sigma interactions between Phe-118 and C-16, while pi-alkyl interactions at the tetracyclic C group and the C-23 site. The presence of Phe-118 indicates the potential for the benzene group of amino acid residues to strongly interact with tomatidine.

Van der Waals interactions, alkyl-alkyl, and pi-alkyl were dominantly observed in the ligand-receptor complex of all target proteins, including JAK1 and SMO. Although the Van der Waals and alkyl interactions are relatively weak, the forces formed by the interaction are likely to support integral structural loads as an increase in the number of atoms that are close together [30,36,37]. The interaction force is formed as a result of the electron density transient shift of the atoms in ligand-receptor binding site [38]. The pi-alkyl interaction was also observed in the ligand-receptor complex of the JAK1 protein formed by Trp-1047 with C-24', then Phe-332 with the C-24' end of the SMO protein.

Ligand-receptor complexes in all target proteins are dominated by non-polar amino acid interactions with the tetracyclic ring structure of tomatidine, and aromatic amino acids with C-17 branching ends. In addition, an average of more than 15 amino acids were involved in the ligand-receptor complex indicating the great potential of tomatidine as a candidate inhibitor of target protein activity. It is relevant to the docking simulation from previous studies that showed the potential of tomatidine as an anti-oncogenic protein activity involved in a multistage of various cancers [7,39-41]. However, the shape of the fit between the ligand and target compounds is influenced by the binding position with the ligand pose, especially in the hydrophobic position. Further analysis is needed to identify formation process of the receptor active site to explain the protein folding mechanism involving phytosterols. This is important considering that the majority of the active sites tend exposed to the environment before inward folding process to facilitate the formation of binding sites.

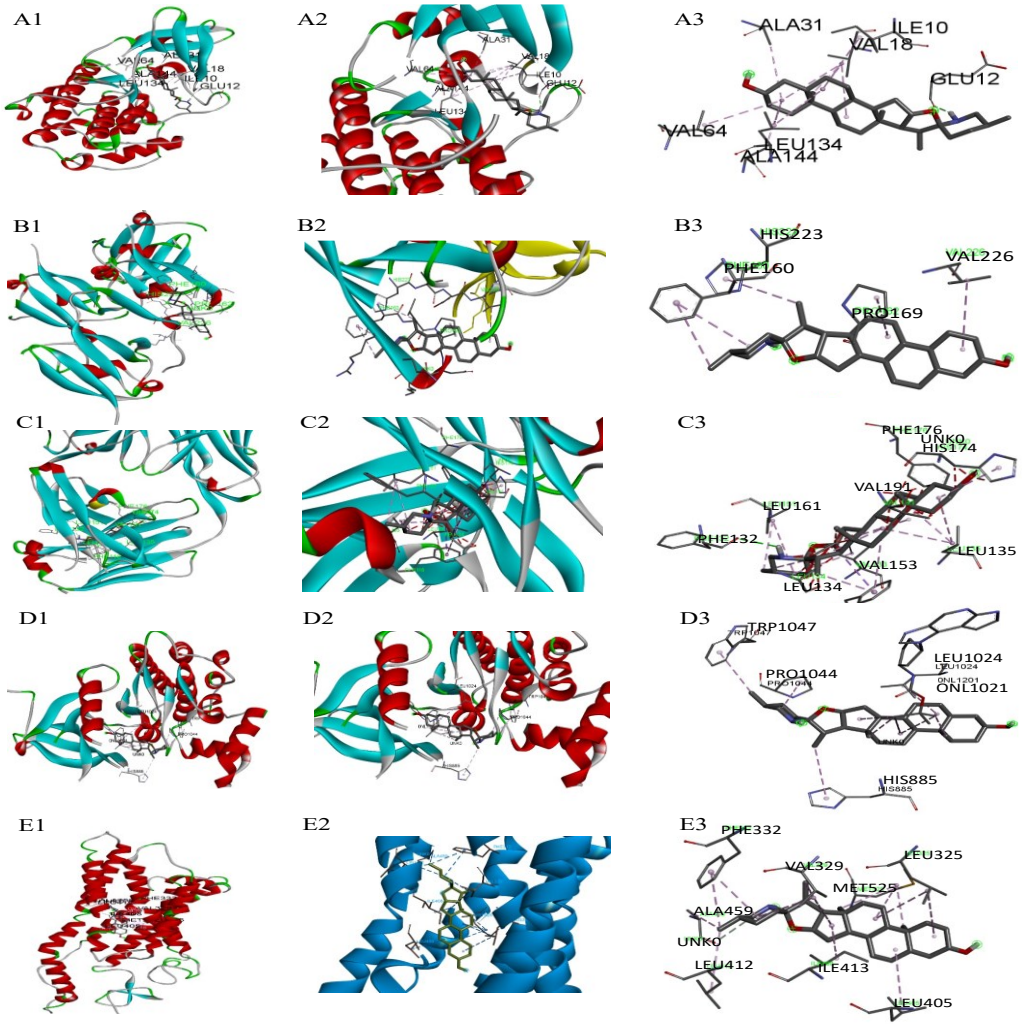

Figure 2 Quaternary 3D structure of target proteins CDK2 (A), BRAF (B), VEGFA (C), JAK1 (D), and SMO (E). The binding site on the target protein is indicated by A2-E2, while the interaction of the ligand with amino acids on the target protein is indicated by A3-E3.

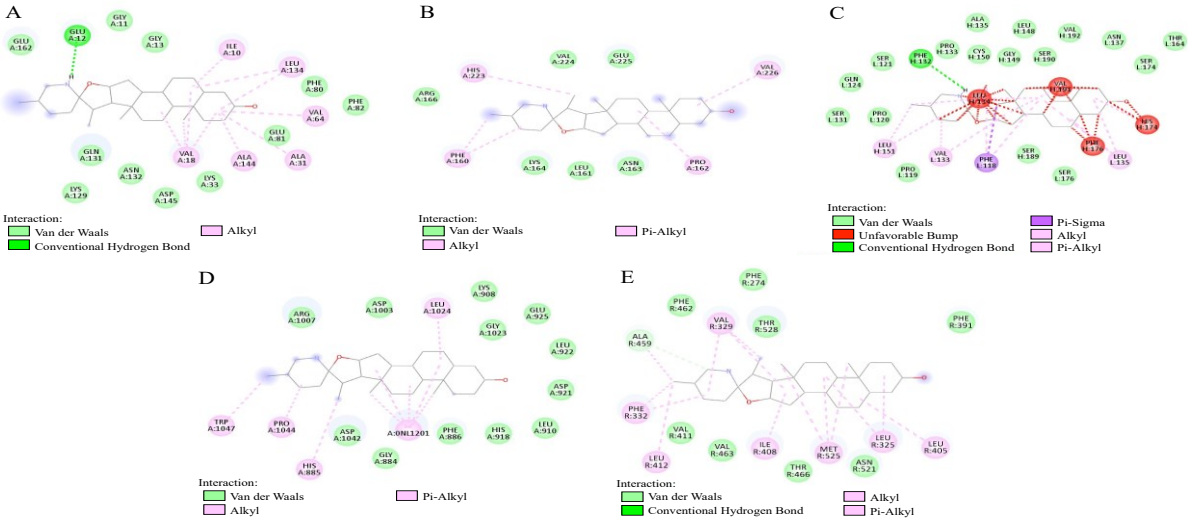

Figure 3 The form of interaction formed between the ligand and amino acid residues at the protein binding sites of CDK2 (A), BRAF (B), VEGFA (C), JAK1 (D), and SMO (E).

Current research explained that tomatidine prevents muscle atrophy through interactions with activating transcription factor 4 (ATF4) (an important mediator in the mechanisms of age-related muscle weakness and atrophy), and reduces aging-associated sarcopenia [42]. Dietary supplementation with 0.04 % tomatidine for 10 weeks has also reduced plasma cholesterol levels and the risk of atherosclerosis [43]. In addition, tomatidine plays a role in influencing the ATF2 gene expression in viral inflammatory response [44]. Tomatidine is also involved in actively upregulating TIMP-1 and RECK, and downregulated MMP-2 in A549 adenocarcinoma cells [27].

Furthermore, based on the docking interaction analysis, tomatidine is potentially developed as a candidate inhibitor for oncogenic protein in cancer cell metabolism. Signaling pathway analysis showed that inactivating CDK2, BRAF, and VEGFA proteins, potentially inhibited the moderation of mitogen-activated protein kinases (MAPK) signaling pathways. JAK1 and VEGFA proteins are involved in the JAK/STAT signaling pathway whereas SMO is involved in a variety of pathways including MAPK, Wnt-signaling, and the Notch cascade [45].

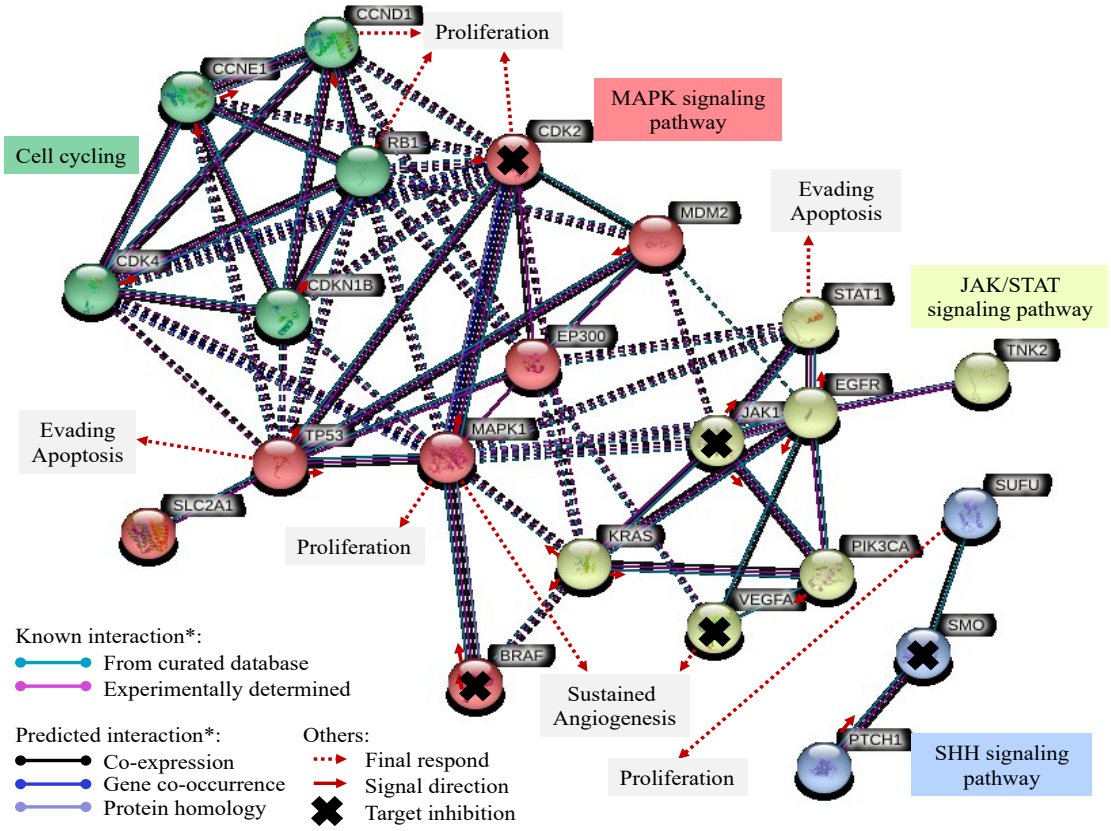

Figure 4 Simulation scheme of target protein signaling for CDK2, BRAF, VEGFA, JAK1, and SMO in several cancer cells' signaling pathways. Color differences indicate clusters of protein involvement in each cascade. The dotted line in the interaction path represents the edges between clusters.

Inactivation of MAPKs signaling pathway has been observed in the synthesis inhibition of matrix metalloproteinases (MMP) proteins and inhibit tumorigenesis [8]. It is known that the MMPs protein family, including MMP-2 and MMP-9, responsible for cancer cell proliferation, migration, and angiogenesis which is regulated during tumorigenesis. Furthermore, the effect of tomatidine in inhibiting BRAF protein potentially inactivates extracellular signal-regulated kinases (ERK) as intermediate factors in the Ras/Raf/MAPKs cascade and prevents cancer cell proliferation. Furthermore, inactivation of ERK also prevents phosphorylation of the necrosis factor kappa B (NF-kB) which inhibits metastasis and invasion of cancer cells. Furthermore, tomatidine supplementation has also been reported to reduce the expression of VEGF and p50 proteins involved in MAPK signaling pathway of the HT1080 fibrosarcoma cell line [8]. Prediction through molecular docking also shows the potential of tomatidine in inhibiting EFGR in lung cancer [46]. Tomatidine also plays a role in inhibiting p53 which is involved in MAPK

signaling and the cell cycle, thereby inhibiting evading apoptosis, sustained angiogenesis, G1/S progression, and metaphase II arrest in cancer [47].

In addition, VEGF has also been involved in the Janus kinase-signal transducer and activator of the transcription (JAK/STAT) signaling pathway. JAK/STAT activation is initiated by phosphorylation of the JAK1 protein which then triggers various types of phosphorylation (STATs) and activates VEGF, which plays a role in sustained angiogenesis of the cancer cell. The presence of activated JAK1 protein enhances the transphosphorylation and autophosphorylation of various proteins associated to the JAK/STAT signaling pathway. The JAK1 activation also promotes tyrosine residues phosphorylation of the STAT protein, then leading simultaneous dimerization and translocation into the cell nucleus. Several studies have shown that inhibition of JAK1 can reduce VEGF function and trigger apoptosis in various types of cancer treated with cucurbitin extract [48], and curcumin [49].

Another dominant cascade for cancer development is the sonic hedgehog (SHH) signaling pathway which is involved in promoting oncogenesis [50,51]. Mutations in SHH can act as the dominant oncogenic ligand because the ectopic expression of SHH (gene expression which it is normally not expressed) increases the frequency of the appearance of carcinoma. In addition, as with other cascade pathways, gene expression and protein activation in the SHH signaling pathway are tightly regulated, especially in adult tissues. This is because overexpression of one of the proteins in the SHH pathway can trigger the progression of various types of cancer. For example, ligand-independent SMO triggers overexpression of unstable SHH and Cyclin-B1 which results in chromosomal instability [52]. Therefore, SMO inhibition is one of the valid and prospective therapeutic targets in inhibiting the growth of various types of cancer [45]. In addition to SMO, recent studies have also shown the dominance of expression of patched homolog 1 (PTCH1), over SMO in lung cancer, so it can be used as a therapeutic target [53,54].

SMO protein inhibitors have now begun to be developed and are relatively effective in inhibiting cancer malignancy and metastasis. Although several studies also explain the failure of SMO inhibition in preventing the growth of several types of cancer due to massive mutations of different genes/chromosomes in different types of cancer. Therefore, the development of anti-SMO drugs needs to be carried out by increasing the drug efficacy through more simulations of docking binding sites [55,56], and followed by *in vitro-in vivo* study. In this study, the development of tomatidine as an inhibitor of SMO protein showed a binding site in the involved hydrophobic area that involved β-sheet structure.

Conclusions

Tomatidine is properly extracted in the chloroform, petroleum ether, and ethanol, respectively, but not in the aquadest. The physicochemical properties of tomatidine provide a closed covalent bond that makes it more insoluble in the aquadest. The only polar side is supported by the hydroxyl group in C-4, side chain C-17, and amide side in N-24 allowing hydrogen bond formation. Further research needs to be developed to determine the best maceration process and solvent concentration to extract tomatidine compounds from fruit.

Tomatidine physicochemical property is relevant with the docking simulation, which highly triggers Van der Waal force than hydrogen bond or ionic force. Moreover, the ligand-receptor interaction makes tomatidine invaginated or folded into the protein hydrophobic side and strongly attracts non-polar and aromatic amino acids, mostly Val, Ala, Leu, and Phe. Tomatidine significantly creates an interaction with targeted proteins, including CDK2, BRAF, VEGFA, JAK1, and SMO that are involved in cancer cell proliferation, evading apoptosis, and sustained angiogenesis. The highest interaction is observed in the SMO-tomatidine interaction complex, which may affect the Sonic Hedgehog (SHH) signaling pathway. This is a significant finding considering that the SMO protein and SHH signaling pathway are one of the most significant oncogene expression pathways that contribute to the emergence of various types of cancer. It makes tomatidine therapeutically potential to inhibit cancer cell proliferation through protein inhibition activities. Further study should be focused on the effectiveness analysis of the SHH signaling pathway in the cancer cell.

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