Babol University of Medical Sciences
IJMCM
International Journal of Molecular and Cellular Medicine
p-ISSN: 2251-9637 o-ISSN: 2251-9645
Cellular and Molecular Biology Research Center

An In Silico Study of Transforming Growth Factor-β Inhibitors: A Potential Target for Diabetic Nephropathy Treatment with Active Compounds from the Active Fraction of Physalis angulata

Ika Rahayu[1,2], Nur Arfian[3,5*], Kris Herawan Timotius[2], Mae Sri Hartati Wahyuningsih[4,5]

1. Doctoral Program of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia.
2. Department of Biochemistry, Faculty of Medicine and Health Sciences, Universitas Kristen Krida Wacana.
3. Department of Anatomy, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia
4. Department of Pharmacology and Therapy, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia.
5. Center for Herbal Medicine, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Article type:
Original Article

Received: 2023.12.09
Revised: 2024.07.04
Accepted: 2024.07.07

ABSTRACT

Transforming growth factor beta (TGF-β) initiates epithelial-mesenchymal transition (EMT) in tubular and glomerular epithelial cells, resulting in excessive production and deposition of extracellular matrix through its interaction with TGF-β receptors, which play a crucial role in TGF-β signaling involving two receptor types, namely TGF-β type I (TβRI) and type II (TβRII). EMT contributes to the pathogenesis of interstitial renal fibrosis, a marker of end-stage kidney disease. This study aimed to identify the bioactive compounds in the active fraction of *P. angulata* and evaluate their ability to inhibit the TGF-β activity and their potential as drug candidates. The active components in the active fraction of *P. angulata* were analyzed using gas chromatography-mass spectrometry (GC-MS). The bioactive compound structures were obtained from the PubChem database, while the protein targets, TβRI and TβRII, were retrieved from the Protein Data Bank (PDB). The molecular docking analyses were performed using PyRx 0.8 and Discovery Studio. SwissADME was used to evaluate ligand properties and druglikeness. Three dominant active compounds were identified, namely palmitic acid, campesterol, and stigmasterol. *In silico* studies demonstrated strong energy bonds existed between TβRI and palmitic acid, campesterol, stigmasterol, and SB431542 with binding energy values of -5.7, -10, -9.4, and -10.9 kcal/mol, respectively. Similarly, they strongly bound to TβRII with binding energy values of -5.2, -7.1, -7.5, and -6.1 kcal/mol, respectively. All compounds meet Lipinski's criteria for druglikeness. Among the identified active compounds, campesterol exhibited the highest affinity for TβRI, while stigmasterol exhibited a strong affinity for TβRII. These findings suggested that the three compounds have potential as drug candidates.

Keywords: Diabetic nephropathy, Transforming growth factor beta, TGF-β inhibitor, *Physalis angulata*

Cite this article: Rahayu I, *et al*. An *In Silico* Study of Transforming Growth Factor-β Inhibitors: A Potential Target for Diabetic Nephropathy Treatment with Active Compounds from the Active Fraction of *Physalis angulata*. *International Journal of Molecular and Cellular Medicine*. 2024; 13(2):234-247.

*Corresponding: Nur Arfian
Address: Department of Anatomy, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia.
E-mail: nur_arfian@ugm.ac.id

© The Author(s).
Publisher: Babol University of Medical Sciences
This work is published as an open access article distributed under the terms of the Creative Commons Attribution 4.0 License (http://creativecommons.org/licenses/by-nc/4). Non-commercial uses of the work are permitted, provided the original work is properly cited.

[Downloaded from ijmcmed.org on 2026-10-24]

[DOI: 10.22088/IJMCM.BUMS.13.3.234]

Introduction

Transforming growth factor beta (TGF-β) is a member of a large family of multifunctional proteins involved in various cellular processes, including cell proliferation, apoptosis, migration, differentiation, and extracellular matrix production (1). To date, five distinct variants of TGF-β (TGF-β1~5) have been identified. Among these, TGF-β1, TGF-β2, and TGF-β3 are primarily found in mammals, with TGF-β being the most prevalent, constituting over 90% of this isoform (2). TGF-β initiates epithelial-mesenchymal transition (EMT) in both tubular and glomerular epithelial cells, resulting in excessive production and deposition of extracellular matrix in the glomeruli and tubulointerstitium (3). EMT is a phenotypic conversion involved in the pathogenesis of interstitial renal fibrosis (4), a marker of end-stage kidney disease. Diabetic nephropathy or diabetic kidney disease is a microvascular complication due to prolonged hyperglycemia characterized by fibrosis (5). This condition has led to the identification of the TGF-β signaling pathway as a promising target for therapeutic intervention.

When TGF-β binds to the serine/threonine kinase receptors known as TGF-β type I (TβRI) and type II (TβRII), it initiates both SMAD-dependent and SMAD-independent signaling pathways (6). The initiation of intracellular signals by TGF-β superfamily ligands occurs through their interaction with two categories of transmembrane receptors: TβRI, also known as activin receptor-like kinase (ALK), and TβRII. These receptors contains a serine/threonine kinase domain. Aberrant TGF-β signaling is associated with various conditions, including fibrosis, cardiovascular diseases, and cancer. TGF-β has long been recognized as a pivotal cytokine in the progression of kidney inflammation and fibrosis (7). The active form of TGF-β engages with TβRII, activating TβRI and downstream receptor-associated SMADs (R-SMADs), specifically SMAD2 and SMAD3. Following phosphorylation, SMAD2 and SMAD3 form a multiunit complex with SMAD4 (8). This SMAD2/3/4 complex translocates to the cell nucleus to regulate the transcription of specific genes. This regulatory process results in the expression of α-smooth muscle actin (α-SMA) and collagens and inhibits SMAD7 (9). SMAD7 can counteract TGF-β-induced fibrosis, carcinogenesis, and inflammation in various diseases (10, 11).

Various organic compounds have been deliberately designed and developed to inhibit the TGF-β signaling pathway. These inhibitors function either by suppressing TGF-β expression or by obstructing the kinase activities of TGF-β receptors (6). Blocking the TGF-ß signaling at the receptor kinase level can halt the biological effects of TGF-ß. Several small compounds have been synthesized to target the ATP binding sites of TβRI or TβRII, thereby preventing the phosphorylation of substrates. Binding to the kinase domain impedes the ability of ALK5 receptor to activate SMAD2/3 (12).

The *P. angulata* herb exhibits significant antioxidant capacity and demonstrates vasoprotective, renoprotective, anti-inflammatory, and antidiabetic properties. This herb has the potential to reduce blood glucose levels, malondialdehyde (MDA), and advanced glycation end-products (AGEs) that are involved in the development of diabetic kidney disease. Additionally, *P. angulata* shows potential for promoting lymphocyte cell proliferation (13, 14, 15, 16).

Molecular docking is frequently employed in bioinformatics research for virtual screening and drug design based on molecular structures. It predicts non-covalent interactions, primarily hydrogen bonding, between a large molecule (receptor) and a smaller molecule (drug). AutoDock is a suitable software program

[Downloaded from ijmcmed.org on 2026-10-24]

[DOI: 10.22088/IJMCM.BUMS.13.3.234]

for conducting docking and virtual screening, as it automatically calculates the necessary grid for atom types (17,18). Binding interactions between receptors and ligands are analyzed using Discovery Studio by BIOVIA. These interactions can identify target compounds related to *in vitro* and *in vivo* activities. *In silico* research also relies heavily on ADMET and druglikeness to explore novel targets and compounds with anticipated biological effects (19).

The active compounds and biological activities of *P. angulata* suggest its potential as a candidate for inhibiting TGF-β activity, which is associated with kidney fibrosis, a marker of diabetic nephropathy. This study aimed to identify the bioactive compounds in the active fraction of *P. angulata* and evaluate their ability to inhibit the TGF-β activity and the potential as drug candidates.

Materials and methods

Sample

The active fraction of *P. angulata* herb extract was obtained from the Department of Pharmacology and Therapeutics, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada. The active fraction was derived from the chloroform extract of *P. angulata.*

Phytochemical Analysis Using GC-MS

The active components in the active fraction of *P. angulata* were analyzed using gas chromatography-mass spectrometry (GC-MS). An Agilent HP-5ms column (30 m x 0.25 mm, 0.25 µm) was used, with helium gas as the mobile phase at a total flow rate of 1 mL/min. The sample, consisting of 50 µL dissolved in 2 mL of ethanol and homogenized using an ultrasonicator for 30 minutes, was injected in a volume of 1 µL. The Willey Library software was employed during the screening process to identify the active substances within the samples.

In silico analysis

Ligand preparation

Predictive models for the primary anti-target TβRI were developed using homology modeling. Subsequently, molecular docking was conducted using the generated models. The molecular docking analyses included all targets and were performed using AutoDock Vina within the PyRx 0.8 software suite. Ligand configurations were obtained from PubChem. The 3D chemical structures of palmitic acid, campesterol, stigmasterol, and SB31542 were acquired in a structured data file (SDF) format. SB31542 was used as a control in this study due to its ability to inhibit the TGF-β-induced EMT (20). The ligand structures underwent energy minimization for optimal stability and were subsequently converted into a Protein Data Bank, Partial Charge, and Atom Type (PDBQT) format using Open Babel within PyRx 0.8.

Protein preparation

The 3D crystal structure of the type I TGF-β receptor (TβRI; PDB ID: 3TZM) and type II TGF-β receptor (TβRII; PDB ID: 1M9Z) were retrieved in a PDB format from the Protein Data Bank (http://www.rcsb.org). Before commencing the docking process, non-protein components, such as water or solvent molecules, were eliminated from the molecular structures. Hydrogen atoms were subsequently added to the protein configuration, and molecular docking was performed using PyRx 0.8.

[Downloaded from ijmcmed.org on 2026-10-24]

[DOI: 10.22088/IJMCM.BUMS.13.3.234]

The active sites were determine using the Discovery Studio Visualizer (DSV) v21.1. For TβRI (3TZM), the active site was found to consist of amino acid residues of Ile211, Gly214, Ala230, Lys232, Leu278, Ser280, Asp281, Tyr282, His283, Lys337, Leu340, and Asp351. This finding was consistent with Ogunjimi *et al.* (21), who found that the native ligand of the receptor interacted with these amino acid residues. Therefore, these compounds were used to determine the active site. Based on the information provided by DSV, the dimensions of TβRI receptor were configured as follows: X-axis of 30.2 Å, Y-axis of 31.6 Å, and Z-axis of 20 Å.

For TβRII (1M9Z), which had no native ligand, the active site was defined manually using DSV. The active site was found to consist of amino acid residues of Asp39, Asn40, Gln41, Trp65, Phe111, and Phe126. Therefore, these compounds were used to determine the active site. Based on the information provided by DSV, the dimensions of TβRII receptor were configured as follows: X-axis of 27.4 Å, Y-axis of 16.8 Å, and Z-axis of 25.5 Å.

Protein-ligand docking

Subsequently, ligand docking was performed using the specified dimensions. Grid boxes were set within PyRx 0.8 to match these dimensions precisely, covering the entire active site of the protein to explore potential interactions between proteins and ligands. The docking simulations were performed using PyRx 0.8, and the resulting complex structures were visualized using DSV.

Druglikeness prediction

Druglikeness prediction was carried out using the SwissADME online platform (http://www. Swiss-adme. ch), adhering to the Lipinski's rule of five. This rule specifies a molecular weight (MW) of no more than 500 Da, H-bond donors of no more than five, a log P of no more than 5, and H-bond acceptors of no more than 10 (22).

Results

Identified compounds

GC-MS was used to identify the bioactive compounds in the active fraction of *P. angulata*. This powerful analytical method combines the features of gas chromatography and mass spectrometry to identify different substances within the active fraction. Each compound generates a distinct mass spectrum, facilitating its identification and characterization through comparison of retention times (RT) and percentage areas. The resulting GC-MS chromatogram data were analyzed using the Willey Library software. Three primary active compounds were identified: palmitic acid, campesterol, and stigmasterol. Table 1 provides a list of all identified compounds in the active fraction of *P. angulata*. These three active compounds were used for further analysis.

Molecular docking studies

Table 1. GC-MS phytochemical analysis results.

Identified compound	m/z	Retention time (RT)	% area	Molecular formula
Palmitic acid	73; 60; 43	19.790	58.1	$C_{16}H_{32}O_2$
Campesterol	207; 43; 55	34.910	39.8	$C_{28}H_{48}O$
Stigmasterol	207; 55; 28	35.594	22.8	$C_{29}H_{48}O$

[Downloaded from ijmcmed.org on 2026-10-24]

[DOI: 10.22088/IJMCM.BUMS.13.3.234]

The interactions between the active compounds (ligands) and TGF-β receptors (TβRI and TβRII) were analyzed by AutoDock Vina within Pyrx 0.8 and Discovery Studio. Table 2 presents the highest docking scores obtained for 3TZM and 1M9Z with the ligands. The results suggested that campesterol exhibited the strongest binding affinity with TβRI among the three ligands, approaching the affinity observed with the TGF-β inhibitor, SB431542. Conversely, stigmasterol exhibited a strong affinity with TβRII. The interactions between the active compounds and TGF-β receptors are illustrated in Table 3 and Figure 1.

Table 2. The highest binding affinity scores of the active compounds and the target proteins.

Active compound	TβRI (kcal/mol)	TβRII (kcal/mol)
Palmitic acid	-5.7	-5.2
Campesterol	-10	-7.1
Stigmasterol	-9.4	-7.5
SB431542	-10.9	-6.1

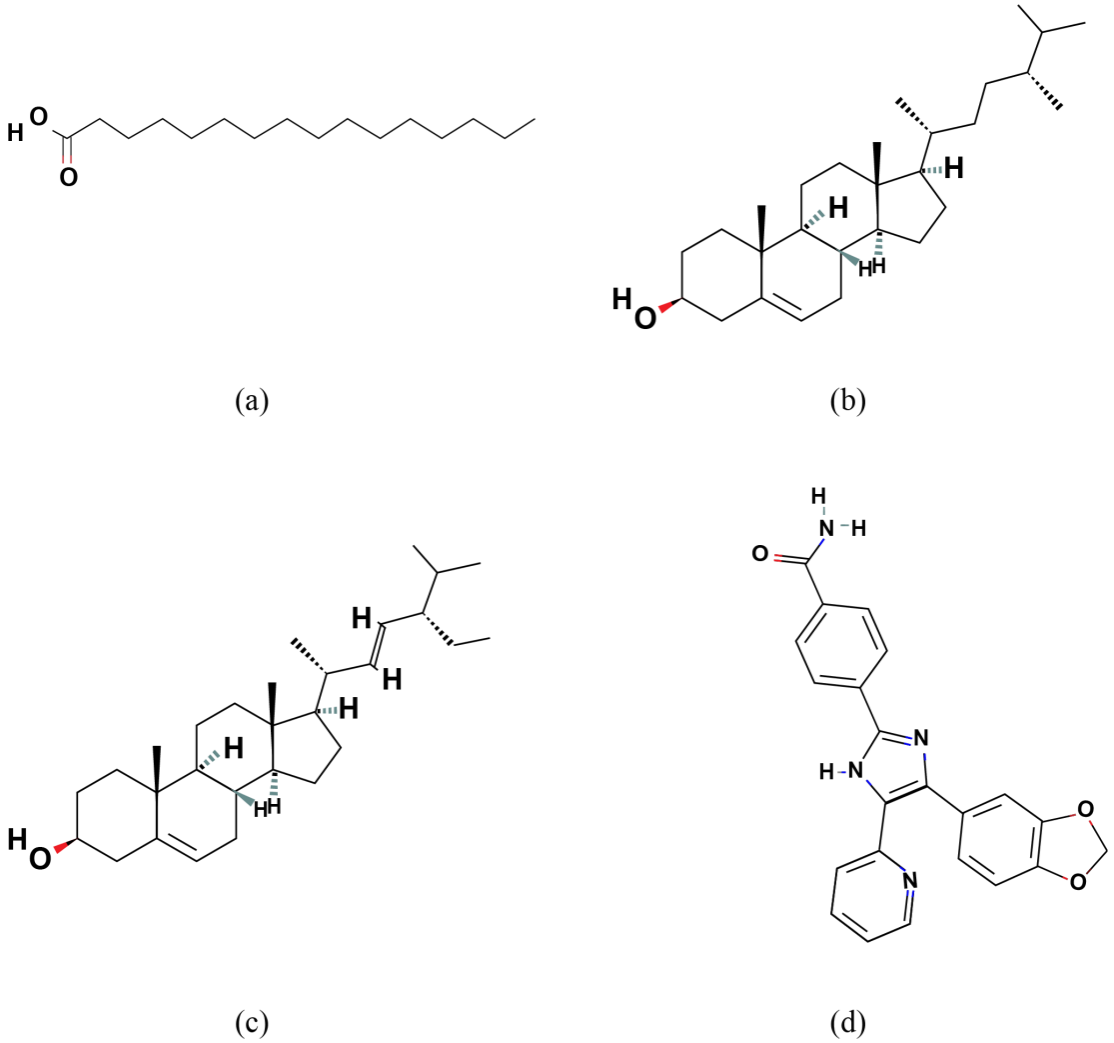


Fig. 1. Structures of the identified compounds and the standard. Palmitic acid (a); Campesterol (b); Stigmasterol (c); and SB431542 (d).

[Downloaded from ijmcmed.org on 2026-10-24]

[DOI: 10.22088/IJMCM.BUMS.13.3.234]

Table 3. Interaction between the active compounds and TGF-β receptors.

Active Compound	Interaction	Residues	
		TβRI	**TβRII**
Palmitic acid	Van der Waals	Glu245, Tyr249, Phe262, Val279, Val231, Asp351, Ile211, Ser287, Lys337, Asn338	Lys101, Ile99, Asn40, Gln41, Thr109, Asp39
	Conventional hydrogen	Leu278, Ser280, Ala230	Glu128, Met100
	Carbon hydrogen	-	Ser127
	Alkyl and pi-alkyl	Lys232, Ala350, Val219, Leu260, Leu340	Phe111, Phe126, Trp65, Lys67
Campesterol	Van der Waals	Ala230, Leu340, Ser287, Thr375, Val231, Ser280, Val279, Leu278, Phe262, Tyr249, Ala350, Glu245, Asn338, Asp351, Lys213, Gly214, Arg215	Ser127, Met100, Glu128, Thr109, Glu102, Asn40, Gln41, Asp39
	Alkyl and pi-alkyl	Leu260, Lys232, Val219, Lys337	Lys101, Phe111
	Pi-S Pi-sigma	-	Trp65, Phe126
	Unfavorable donor-donor	Lys335	-
Stigmasterol	Van der Waals	Leu260, Ala350, Lys232, Asp351, Lys213, Lys337, Asn338, Arg215, Gly214, Lys335, Ser287, Gly286, Glu284, Tyr282, His282	Asp39, Asn40, Gln41, Ser127, Thr109, Lys42
	Carbon hydrogen	-	Glu129
	Pi-sigma	-	Phe126
	Alkyl	Val219, Ala230, Leu340, Ile211	Trp65, Phe111, Lys67
SB431542	Van der Waals	Asp281, Asn338, Lys337, Lys335, Arg215, Asp351, Ala350, Gly214, Glu245, Leu278, Val231, Leu260, Gly286, Ile211	-
	Conventional hydrogen	His283	Lys101, Lys103, Glu91
	Carbon hydrogen	Ala230	-
	Pi-donor hydrogen	Ser280	-
	Pi-sigma	Leu340	-
	Pi-pi stacked	Tyr282	-
	P-alkyl	Val219	Ala94
	Pi-cation	-	Lys101

[Downloaded from ijmcmed.org on 2026-10-24]

[DOI: 10.22088/IJMCM.BUMS.13.3.234]

The ADME assessment

The assessment of absorption, distribution, metabolism, and excretion (ADME) indicated that almost all compounds met the Lipinski's criteria (Table 4). The Lipinski's criteria for good drug absorption and permeation in the body specifies H-bond donors of no more than five, molecular weight of no more than 500 Da, a log P of no more than five, and H-bond acceptors of no more than 10 (20). However, campesterol and stigmasterol deviated slightly from these criteria, especially in their log P values (Table 3). The results of SwissADME assessment suggested that all active compounds from *P. angulata* have the potential for absorption in the human intestine (Table 4).

Table 4. Lipinski's properties of the active compounds in *P. angulata* using SwissADME.

Property	Compound		
	Palmitic acid	**Campesterol**	**Stigmasterol**
MlogP	4.19	6.54	6.62
Log S	-5.31	-5.79	-5.47
TPSA (Å2)	37.30	20.23	20.23
GI Abs	High	Low	Low
MW (g/mol)	254.42	400.68	412.69
Lead-likeness	2	2	2
nHeavy Atoms	18	29	30
Pgp Substrate	No	No	No
Nviolation	1	1	1
NRotB	14	5	5
Synthetic access	2.31	6.17	6.21
Druglikeliness score	-0.54	0.59	0.62
Druglikeness	Yes	Yes	Yes
nHBA	1	1	1
nHBD	1	1	1
Bioavailability score	0.85	0.55	0.55

The ADME assessment demonstrates that the majority of the identified bioactive compounds from *Physalis angulata* adhere to Lipinski's rule of five criteria: **MW**:Molecular weight, indicating the molecular weight of a compound with the recommended range being no more than 500 Da**LogP**: Log of octanol/water partition coefficient, measuring the lipophilicity or hydrophobicity of a compound with the recommended range being no more than 5.**nHBA**: Number of hydrogen bond acceptors, indicating the number of hydrogen bond acceptor atoms in the molecule with the recommended range being no more than 10 .**nHBD**: Number of hydrogen bond donors, indicating the number of hydrogen bond donor atoms in the molecule with the recommended range between 0.0 to 6.0.**nRotB**: Number of rotatable bonds, indicating the number of bonds which allow free rotation around themselves (any single bond, not in a ring, bound to a non-terminal heavy atom) with the recommended range of no more than 10.**Log S**: log of solubility, indicating the solubility of a compound in the water with the recommended value between -9 and 1.5.**TPSA**: Total polar surface area, indicating the polarity of molecules with the recommended range between 20 and 130 Å..**Pgp substrate**: P-glycoprotein substrate in relation to bioavailability and absorption

Discussion

GC-MS is an effective tool for analyzing various volatile and semi- volatile compounds, particularly

[Downloaded from ijmcmed.org on 2026-10-24]

[DOI: 10.22088/IJMCM.BUMS.13.3.234]

those that can be vaporized without decomposition (23). The analysis of the active fraction of *P. angulate* identified three active compounds, namely palmitic acid, campesterol, and stigmasterol.

Discussion

Palmitic acid belongs to the category of saturated fatty acids (24), which is notable for its antibacterial properties (25) and its significant roles in both innate and adaptive immunity (26). It also exhibits antioxidant, anti-inflammatory, hypocholesterolemic, and potential cancer-prevention properties (27).

Campesterol and stigmasterol are major phytosterols found in plants. Phytosterols are known to positively influence human health by lowering plasma cholesterol levels and demonstrating anti-inflammatory, antidiabetic, and anticancer effects (28). Specifically, stigmasterol is found to significantly improve hyperglycemia and hyperlipidemia in mice. It is a promising therapeutic agent for type 2 diabetes mellitus (T2DM) by enhancing the expression and translocation of GLUT4 both in vitro and in vivo (29).

Given the potential of the active fraction of *P. angulata*, this study conducted the molecular docking of its active compounds against the target proteins, namely the TGF-β receptors. Molecular docking is a computational modeling technique for investigating the interactions between protein targets and small ligands (compounds) (30). The docking score or binding energy obtained from this process indicates the binding affinity between the ligand and the target protein (31).

TGF-β is a marker for fibrosis in the development of diabetic nephropathy (2). Using active compounds as TGF-β inhibitors is a novel approach to counteracting TGF-β. This method specifically targets the inhibition of latent TGF-β activation at sites of excessive TGF-β activity (32). Since TGF-β signaling is mediated by cell-surface serine-threonine kinases, blocking TGF-β signaling at the receptor kinase level will eliminate its biological functions (33). SB431542, a potent and recently developed TGF-β inhibitor, was used as a control in this study due to its ability to inhibit the epithelial-mesenchymal transition (EMT), transcription, gene expression, and apoptosis induced by TGF-β (34,20). The results of this study showed that campesterol exhibited the highest affinity with TβRI, while stigmasterol exhibited a strong affinity with TβRII. These findings are comparable to the binding affinity of the TGF-β inhibitor, SB431542 (Table 2). Typically, inhibitors interact with target proteins through electrostatic, hydrogen-bonding, and hydrophobic interactions with amino acid residues near the active site (32). The interactions between palmitic acid and TβRI, as well as TβRII, involve Van der Waals' forces, conventional hydrogen bonds, carbon-hydrogen bonds, alkyl interactions, and pi-Alkyl interactions. Similarly, the interactions between campesterol with TβRI and TβRII involve Van der Waals' forces, alkyl interactions, pi- alkyl interactions, pi- sigma interactions, and unfavorable donor- donor bonds. Conversely, the interactions between stigmasterol and TβRI as well as stigmasterol and TβRII involve Van der Waals' forces, carbon-hydrogen bonds, pi-sigma interactions, and alkyl interactions (Table 3, Figure 2).

Hydrogen bonds are crucial in inhibiting complex molecules, providing essential stability to their structures and functions. Analyzing hydrogen bonds is the most effective way to understand the binding mechanism of a compound within the active site (35). Among the three compounds, only palmitic acid formed conventional hydrogen bonds with TβRI and TβRII. Palmitic acid bound to the amino acid residues of TβRI, namely Leu278, Ser280, and Ala230, and TβRII, namely Glu128 and Met100 (Table 3, Figure 2).

[Downloaded from ijmcmed.org on 2026-10-24]

[DOI: 10.22088/IJMCM.BUMS.13.3.234]

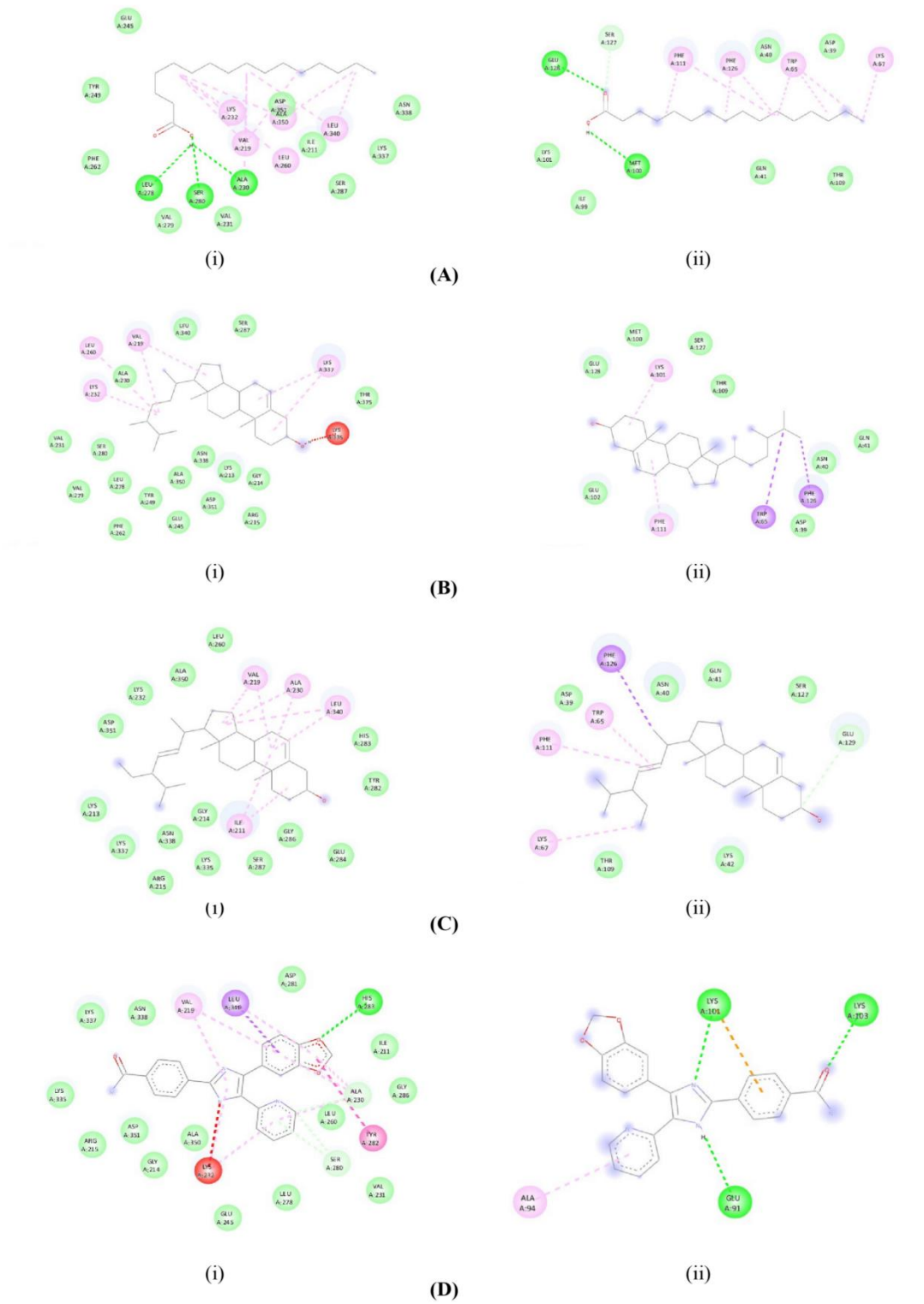


Fig. 2. Results of TGF-β receptors docking with *P. angulata* active compounds. A(i) TβRI-hexadecanoic acid, (ii) TβRII-hexadecanoic acid; B(i) TβRI-campesterol, (ii) TβRII-campesterol; C(i) TβRI-stigmasterol, (ii) TβRII-stigmasterol; D(i) TβRI-SB431542, (ii) TβRII-SB31542

[Downloaded from ijmcmed.org on 2026-10-24]

[DOI: 10.22088/IJMCM.BUMS.13.3.234]

Both palmitic acid and stigmasterol exhibited carbon-hydrogen interactions with amino acid residues of TβRII. Palmitic acid bound to Ser127, while stigmasterol bound to Glu129. Hydrogen bond interactions influence the affinity strength between ligands and amino acid residues. The greater the number of hydrogen bonds formed, the more robust and stable the bond becomes (35, 36).

Although less potent than hydrogen bonds, Van der Waals' forces play a significant role in inhibiting the activity of target receptors and forming stable complexes between ligands and receptors (36). All compounds in this study exhibited Van der Waals' forces. Hydrophobic interactions arising from pi-sigma, alkyl, and pi-alkyl interactions were also present. All compounds exhibited hydrophobic interactions with the receptors. Palmitic acid and campesterol bound to five amino acid residues of TβRI and four amino acid residues of TβRII through alkyl and pi-alkyl interactions (Table 3, Figure 2). Conversely, stigmasterol bound to four amino acid residues of TβRI and three amino acid residues of TβRII through alkyl interactions.

Furthermore, stigmasterol bound to one amino acid residue of TβRII through pi-sigma interactions (Table 3, Figure 2). These interactions could potentially inhibit receptor activity, suggesting their potential utility in designing specific inhibitors (36). Hydrogen bonds and hydrophobic interactions are essential for stabilizing ligands at the target site. Hydrophobic interactions play a central role in the interactions between drugs and receptors (37). Meanwhile, hydrogen bonds are crucial in molecular recognition processes, protein folding stabilization, and the formation and stability of complexes between proteins and ligands (38). These interactions affect both the binding affinity and the effectiveness of the drug.

The aim of predicting druglikeness is to determine if a compound meets the criteria for being considered a drug molecule. The Lipinski's rule of five is a widely adopted method for evaluating the solubility and permeability of a compound, thereby predicting its potential as a drug candidate by assessing its oral effectiveness (35). The Lipinski's rule sets specific thresholds for molecular weight (MW), the number of hydrogen bond acceptors and donors (HBA and HBD), and the water/octanol partition coefficient (log P). Compounds that fail two or more of these criteria are typically excluded from further development. In this study, nearly all compounds met the criteria (Table 4).

Therefore, these three compounds have the potential to qualify as drug candidates with favorable absorption and permeation properties. The Lipinski's rule of five was formulated to establish criteria for the suitability of new molecular entities (NMEs) as drugs. In the context of drug discovery, this rule posits that compounds with more than five hydrogen bond donors, ten hydrogen bond acceptors, a molecular weight exceeding 500, and a calculated log P (clog P) higher than five are likely to exhibit poor absorption or permeation (39).

The ADMET (absorption, distribution, metabolism, excretion, and toxicity) characteristics of the active compounds in *P. angulata* were assessed using the SwissADME. The results indicated that all active compounds could be absorbed in the human intestine (Table 4). The absorption capacity of human intestines is higher for palmitic acid than campesterol and stigmasterol, suggesting that palmitic acid may be more effectively absorbed from the gastrointestinal tract when administered orally (18). SwissADME calculates the clog P (logarithm of the partition coefficient of the compound between water and n-octanol), which determines the hydrophilicity of the compound. The log p- value of palmitic acid is lower than that of campesterol and stigmasterol, although all three compounds fall into the category of moderate solubility

[Downloaded from ijmcmed.org on 2026-10-24]

[DOI: 10.22088/IJMCM.BUMS.13.3.234]

based on their log S values. This finding is supported by the Topological Polar Surface Area (TPSA) values. For optimal polarity, the TPSA should be between 20 and 130 Å (40). TPSA measures the surface area occupied by polar atoms and molecules within the compound. A higher TPSA value is associated with decreased membrane permeability (41). Compounds with elevated TPSA values are more likely to be substrates for P-glycoprotein (Pgp), which expels drugs from cells. Therefore, reduced TPSA values are advantageous for drug-like properties (42). The results of this study indicated that the three active compounds fall within the range of TPSA values and are on the lower end. Furthermore, none of the active compounds were identified as substrates for Pgp, suggesting they are not prone to being expelled from cells by this transporter. This enhances the absorption and bioavailability of the compounds as they are less likely to be actively removed from the cells.

One of the limitations of this study is that it relied on *in silico* methods to computationally predict the capabilities of the three active compounds in *P. angulata*. This study was focused on evaluating the binding affinities of the compounds to TβRI and TβRII, as well as their ADMET properties. While *in silico* methods are valuable, they cannot fully replace the essential *in vitro* and *in vivo* experiments. Therefore, further *in vitro* and *in vivo* studies are needed to assess the potential of these active compounds in modulating the TGF-β signaling pathway and to gain deeper insights into their mechanisms as anti-fibrotic agents. Fibrosis can be modeled *in vitro* by inducing fibroblast cells with TGF-β. Another method involves treating TGF-β-induced fibroblast cells with active compounds derived from the active fraction of *P. angulata.* This research focuses on examining changes in fibrosis markers, such as α-SMA and PDGFR-β, as well as the production of extracellular matrix. *In vivo* studies involve inducing fibrosis in animal models, specifically using techniques like Unilateral Ureteral Obstruction (UUO) and Subtotal Nephrectomy in rats or mice. These models effectively induce fibrosis in the animals. Subsequently, the animals are treated with active compounds from the active fraction of *P. angulata,* and the study evaluates differences in fibrosis markers (such as α-SMA and PDGFR-β), along with the production of extracellular matrix. Additionally, toxicity testing of the active compounds is essential to establish. It is also crucial to recognize that the safety and toxicity profiles of these compounds are significantly influenced by the dosage, method of administration, and overall exposure levels. Comprehensive toxicological studies are required to thoroughly assess the safety of using these active compounds from *P. angulata* as potential drug candidates.

The active fraction of *P. angulata* contains three essential compounds: palmitic acid, campesterol, and stigmasterol. Among these, campesterol exhibited the highest affinity with TβRI, comparable to the TGF-β inhibitor SB31542. On the other hand, stigmasterol exhibited a strong affinity with TβRII compared to other ligands. These three compounds have the potential as drug candidates due to their favorable absorption and permeation properties. Additionally, all compounds met the Lipinski's criteria and can be absorbed in the human intestine.

Acknowledgment

The authors would like to thank Universitas Gadjah Mada for their financial support through the Final Project Recognition (*Rekognisi Tugas Akhir*/RTA) program.

Conflict of Interest

The authors have no conflicts of interest to declare.

[Downloaded from ijmcmed.org on 2026-10-24]

[DOI: 10.22088/IJMCM.BUMS.13.3.234]

References

1. Haque S, Morris JC. Transforming growth factor-beta: A therapeutic target for cancer. Hum Vaccin Immunother 2017;13: 1741-50.

2. Wang L, Wang HL, Liu TT, et al. TGF-Beta as a Master Regulator of Diabetic Nephropathy. Int J Mol Sci 2021;22.

3. Gu YY, Liu XS, Huang XR, et al. Diverse Role of TGF-beta in Kidney Disease. Front Cell Dev Biol 2020;8:123.

4. Sheng L, Zhuang S. New Insights Into the Role and Mechanism of Partial Epithelial-Mesenchymal Transition in Kidney Fibrosis. Front Physiol 2020;11:569322.

5. Suneja M. Diabetic nephropathy and diabetic kidney disease. J Diabetes Mellit 2021;11:359-77.

6. Wang H, Chen M, Sang X, et al. Development of small molecule inhibitors targeting TGF-beta ligand and receptor: Structures, mechanism, preclinical studies and clinical usage. Eur J Med Chem 2020;191:112154.

7. Peng D, Fu M, Wang M, et al. Targeting TGF-beta signal transduction for fibrosis and cancer therapy. Mol Cancer 2022;21:104.

8. Meng XM, Tang PM, Li J, et al. TGF-beta/Smad signaling in renal fibrosis. Front Physiol 2015;6:82.

9. Miyazawa K, Miyazono K. Regulation of TGF-beta Family Signaling by Inhibitory Smads. Cold Spring Harb Perspect Biol 2017;9.

10. Troncone E, Marafini I, Stolfi C, et al. Transforming Growth Factor-beta1/Smad7 in Intestinal Immunity, Inflammation, and Cancer. Front Immunol 2018;9:1407.

11. Zhou G, Sun X, Qin Q, et al. Loss of Smad7 Promotes Inflammation in Rheumatoid Arthritis. Front Immunol 2018;9:2537.

12. Olsen OE, Hella H, Elsaadi S, et al. Activins as Dual Specificity TGF-beta Family Molecules: SMAD-Activation via Activin- and BMP-Type 1 Receptors. Biomolecules 2020;10.

13. Febianti Z, PERMATASARI N, SOEHARTO S. Vasoprotective Effect of Physalis Angulata L. Leaf Water Extract on Kidney Of Nω-Nitro-L-Arginine Methyl Ester-Induced Endothelial Dysfunction Rat Model. Asian J Pharm Clin Res 2019;12:432-7.

14. Tuan Anh HL, Le Ba V, Do TT, et al. Bioactive compounds from Physalis angulata and their anti-inflammatory and cytotoxic activities. J Asian Nat Prod Res 2021;23:809-17.

15. Kusumaningtyas R, Laily N, Limandha P. Potential of ciplukan (Physalis angulata L.) as source of functional ingredient. Procedia Chem 2015;14:367-72.

16. Adewoye EO, Oguntola MA, Ige AO. Anti-oxidative and reno-restorative effects of physalis angulata (whole plant extract) in alloxan-induced diabetic male Wistar rats. Afr J Med Med Sci 2016;45:99-108.

17. Forli S, Huey R, Pique ME, et al. Computational protein-ligand docking and virtual drug screening with the AutoDock suite. Nat Protoc 2016;11:905-19.

18. Srivastava V, Yadav A, Sarkar P. Molecular docking and ADMET study of bioactive compounds of Glycyrrhiza glabra against main protease of SARS-CoV2. Mater Today Proc 2022;49:2999-3007.

19. Abdul-Hammed M, Adedotun IO, Olajide M, et al. Virtual screening, ADMET profiling, PASS prediction, and bioactivity studies of potential inhibitory roles of alkaloids, phytosterols, and flavonoids against COVID-19 main protease (M(pro)). Nat Prod Res 2022;36:3110-6.

20. Davies MR, Liu X, Lee L, et al. TGF-beta Small Molecule Inhibitor SB431542 Reduces Rotator Cuff Muscle Fibrosis and Fatty Infiltration By Promoting Fibro/Adipogenic Progenitor Apoptosis. PLoS One 2016;11:e0155486.

21. Ogunjimi AA, Zeqiraj E, Ceccarelli DF, et al. Structural basis for specificity of TGFbeta family receptor small molecule inhibitors. Cell Signal 2012;24:476-83.

[Downloaded from ijmcmed.org on 2026-10-24]

[DOI: 10.22088/IJMCM.BUMS.13.3.234]

22. Ivanović V, Rančić M, Arsić B, et al. Lipinski's rule of five, famous extensions and famous exceptions. Popular Scientific Article 2020;3:171-7.

23. Mitsui K, David F, Dumont E. LC fractionation followed by pyrolysis GC–MS for the in-depth study of aroma compounds formed during tobacco combustion. Journal of analytical and applied pyrolysis 2015;116:68-74.

24. Aparna V, Dileep KV, Mandal PK, et al. Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. Chem Biol Drug Des 2012;80:434-9.

25. Casillas-Vargas G, Ocasio-Malave C, Medina S, et al. Antibacterial fatty acids: An update of possible mechanisms of action and implications in the development of the next-generation of antibacterial agents. Prog Lipid Res 2021;82:101093.

26. Radzikowska U, Rinaldi AO, Celebi Sozener Z, et al. The Influence of Dietary Fatty Acids on Immune Responses. Nutrients 2019;11.

27. Mazumder K, Nabila A, Aktar A, et al. Bioactive Variability and In Vitro and In Vivo Antioxidant Activity of Unprocessed and Processed Flour of Nine Cultivars of Australian lupin Species: A Comprehensive Substantiation. Antioxidants (Basel) 2020;9.

28. Miras-Moreno B, Sabater-Jara AB, Pedreno MA, et al. Bioactivity of Phytosterols and Their Production in Plant in Vitro Cultures. J Agric Food Chem 2016;64:7049-58.

29. Wang J, Huang M, Yang J, et al. Anti-diabetic activity of stigmasterol from soybean oil by targeting the GLUT4 glucose transporter. Food Nutr Res 2017;61:1364117.

30. Iheagwam FN, Ogunlana OO, Ogunlana OE, et al. Potential Anti-Cancer Flavonoids Isolated From Caesalpinia bonduc Young Twigs and Leaves: Molecular Docking and In Silico Studies. Bioinform Biol Insights 2019;13:1177932218821371.

31. Ononamadu CJ, Ibrahim A. Molecular docking and prediction of ADME/drug-likeness properties of potentially active antidiabetic compounds isolated from aqueous-methanol extracts of Gymnema sylvestre and Combretum micranthum. BioTechnologia (Pozn) 2021;102:85-99.

32. Jiang JH, Deng P. Discovery of New Inhibitors of Transforming Growth Factor-Beta Type 1 Receptor by Utilizing Docking and Structure-Activity Relationship Analysis. Int J Mol Sci 2019;20.

33. Zhang Q, Liu F, Qin L, et al. Characterization of TGFbeta-associated molecular features and drug responses in gastrointestinal adenocarcinoma. BMC Gastroenterol 2021;21:284.

34. Koh RY, Lim CL, Uhal BD, et al. Inhibition of transforming growth factor-beta via the activin receptor-like kinase-5 inhibitor attenuates pulmonary fibrosis. Mol Med Rep 2015;11:3808-13.

35. Ibrahim ZY, Uzairu A, Shallangwa G, et al. Molecular docking studies, drug-likeness and in-silico ADMET prediction of some novel β-Amino alcohol grafted 1, 4, 5-trisubstituted 1, 2, 3-triazoles derivatives as elevators of p53 protein levels. Sci Afr 2020;10:e00570.

36. Tallei TE, Tumilaar SG, Niode NJ, et al. Potential of Plant Bioactive Compounds as SARS-CoV-2 Main Protease (M(pro)) and Spike (S) Glycoprotein Inhibitors: A Molecular Docking Study. Scientifica (Cairo) 2020;2020:6307457.

37. Madushanka A, Moura RT, Jr., Verma N, et al. Quantum Mechanical Assessment of Protein-Ligand Hydrogen Bond Strength Patterns: Insights from Semiempirical Tight-Binding and Local Vibrational Mode Theory. Int J Mol Sci 2023;24.

38. Ferreira de Freitas R, Schapira M. A systematic analysis of atomic protein-ligand interactions in the PDB. Medchemcomm 2017;8:1970-81.

39. Benet LZ, Hosey CM, Ursu O, et al. BDDCS, the Rule of 5 and drugability. Adv Drug Deliv Rev 2016;101:89-98.

40. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep 2017;7:42717.

[Downloaded from ijmcmed.org on 2026-10-24]

[DOI: 10.22088/IJMCM.BUMS.13.3.234]

41. Whitty A, Zhong M, Viarengo L, et al. Quantifying the chameleonic properties of macrocycles and other high-molecular-weight drugs. Drug Discov Today 2016;21:712-7.

42. Ahmed J, II, Abdul Hamid AA, Abd Halim KB, et al. P-glycoprotein: new insights into structure, physiological function, regulation and alterations in disease. Heliyon 2022;8:e09777.

[Downloaded from ijmcmed.org on 2026-10-24]

[DOI: 10.22088/IJMCM.BUMS.13.3.234]