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Evaluation of Wound Healing Effect of Spirulina platensis Bioactive Molecules through a Multiligand in Silico Approach: Targeting TGF-β, TNF-α, VEGFR2, and KEAP1

Agnia Muftiasih, Heli Siti Halimatul Munawaroh*, Siti Aisyah
Study Program of Chemistry, Faculty of Mathematics and Natural Sciences of Education, Universitas Pendidikan Indonesia, Jalan. Dr. Setiabudi No. 229, Bandung 40154, Indonesia
*E-mail corresponding author's: heli@upi.edu

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Abstract

This study evaluates the therapeutic potential of bioactive molecules derived from *Spirulina platensis* such as gallic acid, quercetin, acacetin, and pinocembrin in promoting chronic wound healing using a multiligand in silico approach. This strategy may accelerate drug delivery by efficiently identifying potent candidates. Here, four key protein targets involve in wound healing, transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α), vascular endothelial growth factor receptor 2 (VEGFR2), and Kelch-like ECH-associated protein 1 (KEAP1), were selected based on their pivotal roles in modulating inflammatory responses, tissue proliferation, angiogenesis, and oxidative stress regulation. Molecular docking simulations revealed that the compound combinations of gallic acid–quercetin and acacetin–pinocembrin exhibited the most favorable binding affinities (-13.21 to -15.567 kcal/mol), surpassing the binding energies of native ligands. These combinations demonstrated strong potential to attenuate the overexpression of pro-inflammatory mediators (TGF-β and TNF-α) and to activate the Nrf2 antioxidant pathway through KEAP1 inhibition. Additionally, quercetin and pinocembrin were predicted to modulate VEGFR2-mediated angiogenesis in a controlled manner. Overall, *Spirulina platensis*-derived bioactive displayed promising synergistic and multitarget interactions that could facilitate and accelerate the wound healing process. These findings provide a rational basis for the development of *Spirulina*-based topical therapeutics, although subsequent dynamic behavior and energetic favorability, *in vitro* and *in vivo* validation is required to substantiate their efficacy.

Keywords: Chronic wound healing, Inflammatory, *In silico*, Molecular docking, *Spirulina platensis*

1 Introduction

Chronic wounds such as diabetic ulcers, venous ulcers, and decubitus remain a major clinical challenge due to their prolonged healing, susceptible to infection, and can lead to serious complications such as amputation. The wound healing process consists of four main phases: hemostasis, inflammation, proliferation, and remodeling [1]. First, the hemostasis phase, which stops bleeding through the formation of platelet plugs and an initial fibrin matrix. Second, the inflammatory phase, in which neutrophils and later macrophages are present to clear damaged tissue and prevent infection. Third, the proliferation phase, which is characterized by the migration of keratinocytes to close the wound, the re-formation of blood vessels (angiogenesis), as well as the formation of granulation tissue by fibroblasts. At this stage, macrophages and regulatory T cells are also involved in the regeneration process. Finally, the remodeling phase involves matrix rearrangement by fibroblasts, regression of new blood vessels, and wound contraction by myofibroblasts to complete healing [1]. Disruption of any of these phases, especially prolonged inflammation and uncontrolled oxidative stress, often causes wounds fails to heal and lead to chronic conditions [1].

Several key proteins play a pivotal role in regulating these phases, including Transforming Growth Factor-beta (TGF-β), Tumor Necrosis Factor-alpha (TNF-α), Vascular Endothelial Growth Factor Receptor 2 (VEGFR2), and Kelch-like ECH-associated protein 1 (KEAP1). TGF-β is

a multifunctional cytokine that regulates tissue proliferation and remodeling, but over expression can lead to hypertrophic scar formation [2]. TNF-α is required for the initial immune response, but chronically high levels can cause apoptosis and inhibit fibroblast function [3]. VEGFR2 is important in angiogenesis to support the supply of oxygen and nutrients to the wound area [4]. Meanwhile, KEAP1 acts as a repressor of the transcription factor Nrf2, which regulates antioxidant genes; KEAP1 inhibition will increase Nrf2 activity and accelerate the wound re-epithelialization process [5].

Recent studies have shown that natural compounds have great potential in regulating this molecular pathway. *Spirulina platensis*, a blue-green microalgae rich in active compounds such as polyphenols, flavonoids, and phycocyanin, has been shown to have strong anti-inflammatory and antioxidant activities [6,7]. Nano phytosome formulations of Spirulina have even been shown to accelerate wound closure, increase collagen deposition, and reduce inflammatory cytokine expression in animal models [8]. Several important flavonoids in Spirulina such as quercetin, gallic acid, acacetin, and pinocembrin have potential as wound healing agents through various mechanisms. Quercetin is able to increase fibroblast proliferation, reduce TNF-α, and regulate TGF-β and VEGF expression [9]. Gallic acid is known to suppress the TGF-β1 pathway and prevent scar tissue formation by inhibiting collagen contraction [10]. Acacetin improves wound healing through activation of the SIRT6/AMPK pathway which reduces oxidative stress and inflammation [10]. Meanwhile, pinocembrin regulates the expression of inflammatory cytokines through the MAPK/NF-κB pathway and supports reepithelialization [11].

In silico approaches, such as molecular docking and virtual screening, have become important tools in modern drug discovery, including for chronic wound therapy. These methods allow for rapid and efficient identification of bioactive compounds that can interact with specific molecular targets involve ed in the wound healing process. For example, a study by [12] used an *in silico* approach to identify natural compounds that mimic HIF-1α, which plays a role in accelerating diabetic wound healing through stabilizing transcription factors important in angiogenesis and cellular metabolism. Similarly, a study by [13] conducted an *in silico* screening of phytomolecules from the ZINC database, finding compounds that showed high affinity for targets such as TNF-α, FGF, and TGF-β, all of which play important roles in the inflammatory and proliferative phases of wound healing. This approach not only accelerates the process of candidate compound discovery but also reduces the need for time-consuming and costly laboratory trials. Thus, the integration of *in silico* methods into chronic wound healing research offers great potential for developing more effective and efficient therapies. *In silico* approaches such as molecular docking are efficient approaches to quickly and accurately select and predict compound interactions with target proteins. Several studies have utilized this method to assess the ability of polyphenolic compounds to bind KEAP1, thus allowing the activation of Nrf2 and the enhancement of the antioxidant response [2]. In addition, docking has also been used to evaluate the interaction of flavonoids with VEGFR2 and TGF-β in the context of wound healing [14].

Although there is abundant evidence regarding the effectiveness of Spirulina in wound healing from experimental studies, few studies have systematically integrated *in silico* approaches to evaluate its interaction with key molecular targets such as TGF-β, TNF-α, VEGFR2, and KEAP1. Current chronic wound therapy is still dominated by the use of growth factors, synthetic anti-inflammatory agents, and bioactive dressings, but are often expensive, non-specific, and risky of side effects. Therefore, there is an urgent need to explore natural, multi-effect, and more affordable therapies [15]

Chronic wound healing remains a clinical challenge due to its multifactorial pathophysiology involving dysregulation of multiple molecular pathways. Despite increasing interest in phytocompounds as multitarget agents, most existing *in silico* studies have focused on limited targets or compound sets, lacking integrative analysis across key proteins involved in wound repair. A study conducted by Thomas *et al.* [13] evaluated plant-derived compounds against TNF-α, FGF, and TGF-β, while another study investigated propolis compounds targeting MMP1 and MMP2 [16]. However, no prior study has systematically explored the simultaneous interaction of multiple bioactive compounds with four major protein targets relevant to wound healing in the context of *Spirulina platensis*-based therapy. This study addresses that gap by evaluating quercetin, gallic acid, acacetin, and pinocembrin bioactive molecules associated with *Spirulina platensis* through a comprehensive

multitarget in silico approach. The simultaneous targeting of four wound-related proteins by a marine microalga-derived compound set represents a novel contribution to the development of sustainable and multifunctional therapeutics. By revealing potential mechanisms of action at the molecular level, the findings provide a scientific rationale for further *in vitro* and *in vivo* validation, and inform the formulation of microalgae-based topical treatments for complex wounds.

2 Method

2.1 Target Protein Preparation

Four major target proteins involved in the chronic wound healing process, namely Transforming Growth Factor-beta (TGF-β), Tumor Necrosis Factor-alpha (TNF-α), Vascular Endothelial Growth Factor Receptor 2 (VEGFR2), and Kelch-like ECH-associated protein 1 (KEAP1), were used in this study. The three-dimensional structures of the proteins were obtained from the Protein Data Bank (https://www.rcsb.org/) [17] with the following PDB IDs: 6B8Y (TGF-β), 2AZ5 (TNF-α), 3WZE (VEGFR2), and 4IQK (KEAP1). Protein preprocessing was performed using PyMOL (Schrödinger L.L.C, 2024), by removing water molecules and adding polar hydrogen atoms. Meanwhile, the process of converting the structure into PDBQT format and adding Gasteiger loads to stabilize the structure [18] was carried out using Autodock Vina 1.2.0 [19].

2.2 Ligand Selection and Preparation

The bioactive compounds tested included quercetin, gallic acid, acacetin, and pinocembrin, along with two compound combinations: gallic acid – quercetin and acacetin – pinocembrin. The gallic acid-quercetin pair was selected for its pharmacodynamic synergy and mutual enhancement of antioxidant, anti-inflammatory, and angiogenic effects essential for wound healing [20,21]. The acacetin-pinocembrin pair, sharing a flavone-flavanone backbone, exhibits structural complementarity and cooperative modulation of MMPs and inflammatory mediators, supporting their use as a multitarget combination [22,23].

The compound structures were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) [24] and converted to PDB format using Open Babel [25] and PDBQT format via Autodock Vina 1.2.0. The compound combination was created by combining two ligands into one molecular docking system via multiple ligands docking in Autodock Vina 1.2.0 to simulate possible synergism in one active site of the protein.

2.3 Molecular Docking Simulation

Docking simulations were performed using AutoDock Vina 1.2.0 software [19] using single ligand docking and multiple ligand docking. The grid box was set around the active site based on the position of the native ligand for each receptor, considering the location of the catalytic residues with a spacing of 0.375 Å. The grid box settings consisting of the coordinates and box sizes can be seen in **Table 1**. Before docking the test ligand, method validation was carried out by re-docking the native ligand into each protein and calculating the Root Mean Square Deviation (RMSD) value, with acceptance criteria if RMSD <2.0 Å, according to general docking validation standards.

Table 1 Coordinates of grid box of each protein receptor for wound healing screening

No	Protein	Coordinate			Size (Å)		
		X	Y	Z	X	Y	Z
1	TGF-β	4.948	8.066	6.368	32	28	32
2	TNF-α	-19.41	74.651	33.85	30	30	30
3	VEGFR2	21.568	25.809	33.177	42	32	42
4	KEAP1	-46.972	3.444	-12.611	32	34	28

2.4 Binding Afiinity and Interaction Analysis

Binding affinity values were obtained in kcal/mol from the simulation results, with the best affinity determined by the most negative binding energy value. The protein-ligand complex with the best affinity was further analyzed using Biovia Discovery Studio Visualizer (Dassault Systèmes BIOVIA, 2021). Interaction evaluation includes identification of hydrogen bonds and hydrophobic interactions to assess the potential biological activity of the compound against the protein target [26].

3 Result and Discussion

3.1 Data on native ligand interactions with receptors

Re-docking of native ligands is necessary to validate the docking method and as a comparison with the target. RMSD analysis between native ligands before and after docking is shown in **Table 2**. The superimposed visualization between native ligands before and after docking is shown in Figure 1. The accepted RMSD value is less than 2 Å [14] and the results show that all have RMSD values of less than 2 Å. This indicates that the docking simulation parameters can be used for the target compound.

Table 2 Validation of RMSD values against native ligands

No	Protein	RMSD Value (Å)
1	TGF-β	0,797
2	TNF-α	0.716
3	VEGFR2	0.677
4	KEAP1	0.953

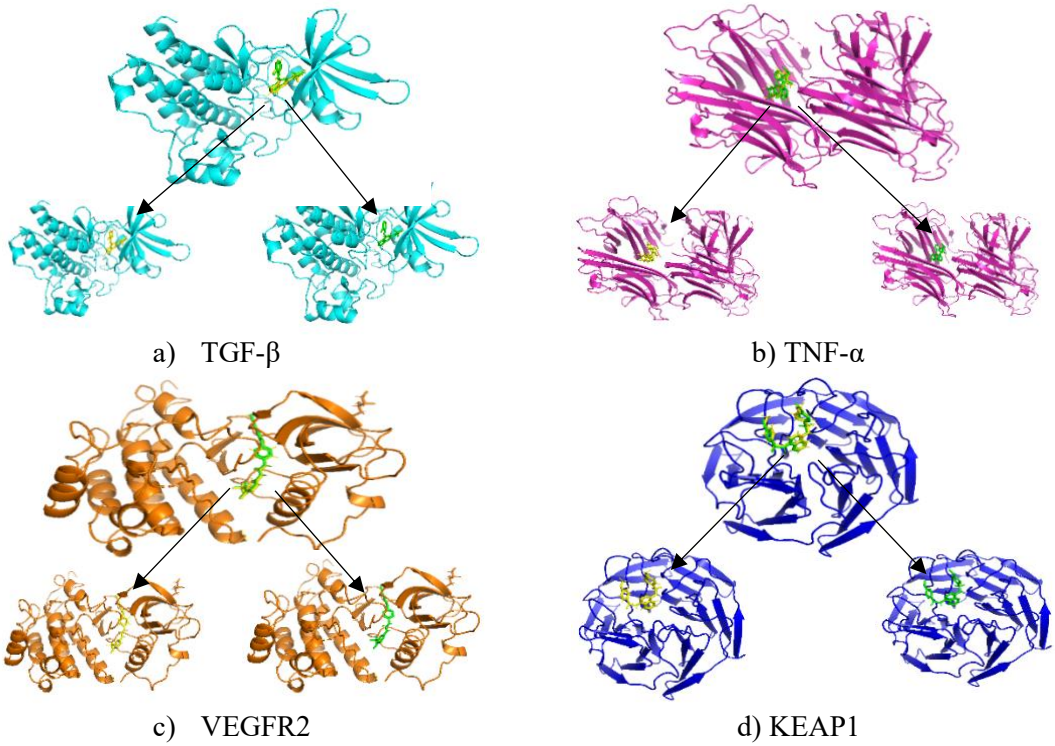

Figure 1 Superimposed visualization of native ligands before and after docking at the active sites of TGF-β, TNF-α, VEGFR2, and KEAP1 (yellow: native ligands before docking, green: native ligands after docking)

High-resolution overlays of native and redocked ligands have been added for each receptor (TGF-β, TNF-α, VEGFR2, and KEAP1). These superimposed visualizations are presented in **Fig. 1a–d**, which illustrate the alignment of ligand poses within the active sites, confirming minimal deviation and appropriate pose conservation.

To enhance readability and facilitate comparative interpretation, the binding affinity data from Table 3 were reorganized into four subtables (**Table 3a-d**), each representing a distinct receptor (TGF-β, TNF-α, VEGFR2, and KEAP1). In addition, a heat map (**Fig. 2**) was included to visually communicate the relative strength of ligand-receptor interactions. The use of color gradients allows clearer visualization of affinity differences among single and combined ligands. This format improves interpretability and aligns with common data visualization practices in computational pharmacology [27]. Based on the *in silico* simulation results shown in **Table 3**, the combination of bioactive compounds from *Spirulina platensis*, namely gallate-quercetin and

acacetin-pinocembrin, showed the most negative binding energy or affinity to the four main receptors in chronic wound healing, namely TGF-β, TNF-α, VEGFR2, and KEAP1. This indicates that both combinations have strong and stable binding affinities, even surpassing native ligands, so they have the potential as multi-target therapy candidates. The strong interaction with TGF-β by gallate and quercetin indicates the potential for inhibition of excessive TGF-β, which is known to affect keratinocyte activity and inhibit re-epithelialization in chronic wounds [2]. Quercetin itself has been shown to be able to stimulate keratinocyte proliferation and normalize TGF-β and VEGF in the wound healing process [28], while gallate is able to inhibit excessive collagen and abnormal fibroblast proliferation during wound healing [29]. Despite its limitations, multiple ligands docking remains a valuable approach for early-stage exploration of compound synergy and potential multitarget activity. It enables rapid screening of candidate molecules against biologically relevant targets, providing structural hypotheses about cooperative binding interactions. This approach is especially useful when prioritizing compounds for downstream validation, offering cost-effective and time-efficient guidance for experimental studies. Previous study demonstrated that multi-ligand docking can uncover novel binding poses and predict cooperative interactions even without accounting for full protein flexibility, making it a pragmatic tool for hypothesis generation in multitarget drug design [30].

Table 3a Binding Affinity score of target compound against receptor TGF-β

Receptor	Native ligand	Test ligand	Binding affinity (kcal/mol)
TGF-β	N-(3-Fluoropyridin-4-Yl)-2-[6-(Trifluoromethyl)Pyridin-2-Yl]-7H-Pyrrolo[2,3-D]Pyrimidin-4-Amine	Native ligand	-11.73
		Gallic acid	-6.71
		Quercetin	-9.436
		Acacetin	-9.701
		Pinocembrin	-9.401
		Gallic acid - Quercetin	-13.87
		Acacetin - Pinocembrin	-15.3

Table 3b Binding Affinity score of target compound against receptor TNF-α

Receptor	Native ligand	Test ligand	Binding affinity (kcal/mol)
TNF-α	6,7-Dimethyl-3-[(Methyl{2-[Methyl({1-[3-(Trifluoromethyl)Phenyl]-1h-Indol-3-Yl}Methyl)Amino]Ethyl}Amino)Methyl]-4h-Chromen-4-One	Native ligand	-9.073
		Gallic acid	-5.716
		Quercetin	-7.281
		Acacetin	-7.554
		Pinocembrin	-7.871
		Gallic acid - Quercetin	-11.52
		Acacetin - Pinocembrin	-14.35

Table 3c Binding Affinity score of target compound against receptor VEGFR2

Receptor	Native ligand	Test ligand	Binding affinity (kcal/mol)
VEGFR2	4-{4-[({[4-Chloro-3-(Trifluoromethyl)Phenyl]Amino}Carbonyl)Amino]Phenoxy}-N-Methylpyridine-2-Carboxamide	Native ligand	-12.88
		Gallic acid	-6.007
		Quercetin	-9.212
		Acacetin	-9.025
		Pinocembrin	-9.294
		Gallic acid - Quercetin	-13.21
		Acacetin - Pinocembrin	-15.57

Table 3d Binding Affinity score of target compound against receptor KEAP1

Receptor	Native ligand	Test ligand	Binding affinity (kcal/mol)
KEAP1	N,N'-Naphthalene-1,4-Diylbis(4-Methoxybenzenesulfonamide)	Native ligand	-10.27
		Gallic acid	-6.707
		Quercetin	-9.212
		Acacetin	-9.008
		Pinocembrin	-9.036
		Gallic acid - Quercetin	-13.45
		Acacetin - Pinocembrin	-15.33

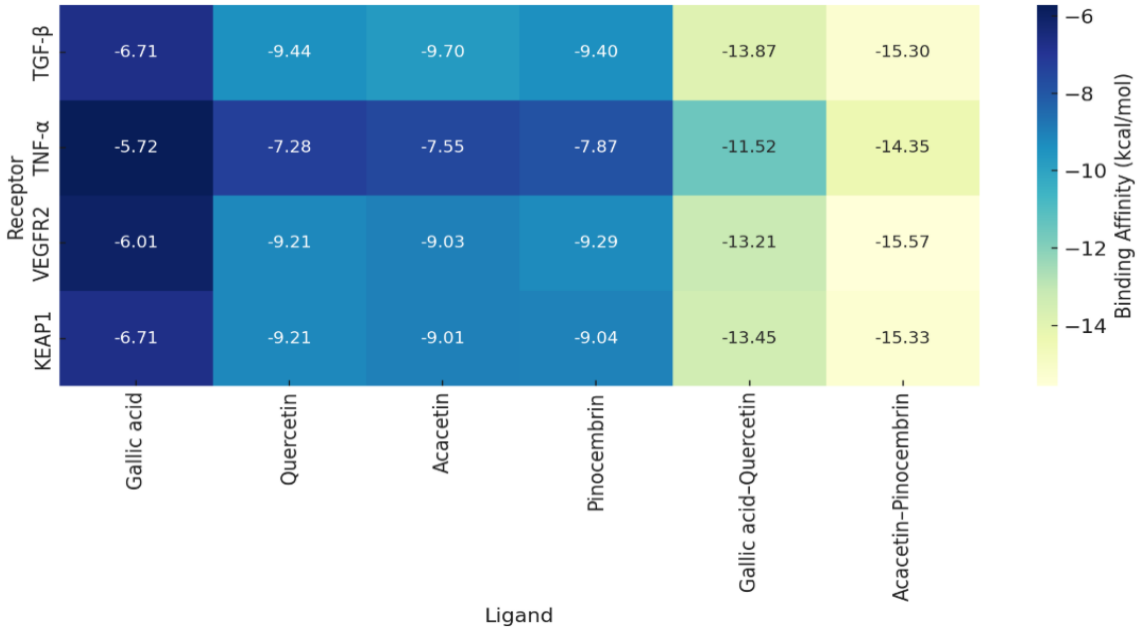

Figure 2 Binding Affinity Heatmap of *Spirulina platensis*-derived molecules to wound healing receptors

Thus, while caution is necessary in interpreting affinity scores, the method still offers significant predictive power in guiding structure–activity relationships and identifying promising compound combinations for further investigation. On the other hand, the interaction of acacetin - pinocembrin with TGF-β shows the potential for inhibition of excessive pro-inflammatory cytokines, considering that pinocembrin has been experimentally shown to suppress the expression of TGF-β1/Smad and attenuate the activation of skin fibroblasts caused by TGF-1[31]. In addition, pinocembrin can suppress the expression of TNF alpha, TNF-α, IL-1β, NO and PGE2 in the inflammatory process [32]. Acacetin is able to increase the endogenous antioxidant response through the activation of the SIRT6/AMPK pathway.

Against VEGFR2, both combinations show high affinity that has the potential to modulate angiogenesis, which is very important in the early and middle phases of wound healing. While these results suggest stronger predicted receptor-ligand interactions, it is important to emphasize that the current analysis is limited to scoring functions derived from docking simulations and does not incorporate entropic contributions or explicit energetic decomposition. Thus, we interpret these results as indicative of enhanced binding potential rather than conclusive evidence of synergistic binding. Without molecular dynamics simulations or free energy analyses, it remains uncertain whether the observed binding improvements stem from additive effects or cooperative molecular interactions. This methodological limitation is common in docking-based studies and warrants

cautious interpretation of "multi-ligand synergy" claims. Nevertheless, the spatial overlap and expanded surface contacts observed in the ligand-receptor complexes provide structural rationale for future enthalpy-entropy-based validation. Quercetin has also been reported in the right concentration to support the migration and differentiation of endothelial cells and accelerate neovascularization, although the agonistic or antagonistic properties still need to be confirmed through further biological tests. The strong interaction of both combinations against KEAP1 is also very important because it indicates the possibility of activating the Nrf2 pathway, which plays a role in the expression of antioxidant genes such as Ho-1 and NQO1 [33].

In addition to binding affinity, the interaction between the receptor and the test ligand can also be influenced by hydrogen and hydrophobic bonds. Based on **Table 4a-d**, the combination of almost all multi-ligands shows more hydrogen bonds to the four receptors than single ligands and shorter bond distances. Likewise with hydrophobic interactions, the presence of more than one ligand can increase the number of these interactions in almost all receptors. This interaction strengthens the stability of the receptor-ligand complex because it plays a major role in optimal ligand docking at the active site of the protein. Thus, the high affinity of the combination of gallate-quercetin and acacetin-pinocembrin to the four main receptors shows that this compound has very promising multi-target potential in chronic wound therapy. Although this study did not include formal energy decomposition or ligand efficiency calculations, the extensive interaction profiling (**Table 4a-d**) provides valuable insight into the molecular determinants of binding stability and specificity. The strong presence of hydrogen bonding and hydrophobic contacts observed across all receptor–ligand complexes suggest favorable anchoring of the compounds within the active sites. Furthermore, all selected bioactive molecules (quercetin, gallic acid, acacetin, and pinocembrin) have been reported compliance with drug-likeness criteria, including Lipinski's Rule of Five [34]. These structural and pharmacokinetic properties support their relevance as potential lead candidates for further preclinical investigation. A more detailed assessment of enthalpic contributions and ligand efficiency indices will be pursued in future studies to more precisely quantify their therapeutic viability.

Fig. 3 and 4 illustrate the structural superimposition of native ligands and the tested multi-ligand combinations (gallic acid–quercetin and acacetin–pinocembrin) across all four wound healing targets. The overlays demonstrate a strong spatial convergence between the test ligands and the respective native ligands at the active sites of TGF-β, TNF-α, VEGFR2, and KEAP1. This suggests accurate pose prediction and validates the docking reliability, in line with the RMSD values reported earlier (<2.0 Å). Such visual validation is particularly critical in multi-ligand docking contexts, where binding site overlap ensures proper modeling of cooperative binding rather than unintended steric clashes [35].

In **Fig. 3**, the gallic acid-quercetin complex shows favorable positioning at overlapping hydrophobic cavities and polar anchor points, particularly within the VEGFR2 and KEAP1 receptors. This confirms that both ligands occupy compatible binding subsites, possibly enabling dual engagement of key residues. Similarly, in **Fig. 4**, acacetin-pinocembrin demonstrates close mimicry of native ligand orientation in TGF-β and TNF-α, which may contribute to enhanced stabilization of the ligand-receptor complex through complementary non-covalent interactions. These structural overlays align with current best practices in structure-based drug design, where superposition of ligand poses provides interpretive support for multitarget hypotheses [36, 37].

Moreover, the observed alignment reinforces the hypothesis that multi-ligand occupancy does not displace the molecule from the catalytically relevant region, but instead augments the interaction network within the active pocket. While these results are promising, future incorporation of ensemble docking or molecular dynamics is necessary to account for receptor flexibility and solvent effects [38]. Therefore, we interpret these overlay results as supporting, but not definitively proving the cooperative binding potential of *Spirulina platensis*-derived compound pairs.

Table 4a. Molecular Interaction of receptor TGF-β and ligand candidates

Receptor	Ligand	Amino acid residue	Category	Total
TGF-β	Gallic Acid	LYS232, SER241, PHE216, ASP351	Hydrogen bond	4
		GLY353, PHE216, GLY353, LEU345, ARG215	Hydrophobic	5
	Quercetin	SER280	Hydrogen bond	1
		VAL219, LEU260, LEU340, ILE211, VAL219, ALA230, LYS232	Hydrophobic	7
	Acacetin	LYS232, SER278, ASP351, ALA230, ASP290, SER280	Hydrogen bond	6
		LEU260, LEU340, VAL219, ALA230, LYS232, ALA350	Hydrophobic	6
	Pinocembrin	LYS232, ASP351, GLU245, ALA230, SER280	Hydrogen bond	5
		LEU260, LEU340, VAL219, ALA230, LYS232, ILE211, VAL219	Hydrophobic	7
	Gallic acid - Quercetin	LYS232, TYR249, ASP351, SER241, GLU245, PHE216, ASP351, SER280	Hydrogen bond	8
		LEU260, GLY353, PHE216, GLY353, LEU354, ARG215, VAL219, ALA230, LYS232, ALA350, VAL216	Hydrophobic	11
	Acacetin-Pinocembrin	LYS213, LEU278, ASP281, SER280	Hydrogen bond	4
		ILE211, LEU340, ALA230, LEU260, HIS283, VAL219, LYS232, LEU278, ALA350	Hydrophobic	9

Table 4b Molecular Interaction of receptor TNF-α and ligand candidates

Receptor	Ligand	Amino acid residue	Category	Total
TNF-α	Gallic Acid	ARG82	Hydrogen bond	1
		LEU93	Hydrophobic	1
	Quercetin	TYR151, LEU120	Hydrogen bond	2
		TRY119	Hydrophobic	1
	Acacetin	TYR151, SER60	Hydrogen bond	2
		TRY59, LEU120, GLY121, TYR151	Hydrophobic	4
	Pinocembrin	-	Hydrogen bond	-
		LEU57	Hydrophobic	1
	Gallic acid - Quercetin	TYR151, SER60, GLN61, GLY121	Hydrogen bond	4
		LEU57	Hydrophobic	1
	Acacetin - Pinocembrin	-	Hydrogen bond	-
		TYR559, TYR119, HIS15, TYR59, TYS151	Hydrophobic	5

Table 4c Molecular Interaction of receptor VEGFR2 and ligand candidates

Receptor	Ligand	Amino acid residue	Category	Total
VEGFR2	Gallic Acid	CYS919	Hydrogen bond	1
		LEU1035, LEU840, VAL848, VAL866	Hydrophobic	4
	Quercetin	LYS868, CYS919, GLUE885	Hydrogen bond	3
		LEU840, LEU1035, PHE918, LEU840, VAL 848, ALA866, LYS868, VAL916	Hydrophobic	9
	Acacetin	LYS868, CYS919, LUE980	Hydrogen bond	3
		LUE1035, LYS868, VAL899, LEU840, VAL848, ALA866, VAL916	Hydrophobic	7
	Pinocembrin	CYS919	Hydrogen bond	1
		LEU840, PHE918, ALA866, VAL848, LYS868, VAL916, CYS1045	Hydrophobic	7
	Gallic acid - Quercetin	CYS919, ASN923, ARG105, ALA105, GLU917, GLY841	Hydrogen bond	6
		LEU840, LEU1035, PHE918, PHE1047, LEU840, ALA866, VAL848, LYS868, VAL916, CYS1045	Hydrophobic	10
	Acacetin - Pinocembrin	CYS919, ARG919, ILE1044, LEU840, GLU815, PHE918	Hydrogen bond	6
		LEU840, ILE888, PHE918 ALA866, VAL848, LYS868, VAL916	Hydrophobic	7

Table 4d Molecular Interaction of receptor KEAP1 and ligand candidates

Receptor	Ligand	Amino acid residue	Category	Total
KEAP1	Gallic Acid	GLY367, VAL512, ILE559, GLY464	Hydrogen bond	4
		ALA366	Hydrophobic	1
	Quercetin	VAL418, VAL465, VAL463, VAL418, VAL606, GLY417	Hydrogen bond	6
		-	Hydrophobic	-
	Acacetin	VAL512, GLY464	Hydrogen bond	2
		ALA556, ARG415, ALA366, ALA556	Hydrophobic	4
	Pinocembrin	GLY367, VAL512, VAL606, GLY367, GLY464	Hydrogen bond	5
		ALA366, ARG415	Hydrophobic	2
	Gallic acid - Quercetin	GLY367, VAL465, VAL512, ILE559, VAL606, ILE559, LEU557, VAL465, GLY364, ILE416, GLY364, GLY511, GLY603, GLY605	Hydrogen bond	14
		ALA556, ILE559, ARG415, ALA556, ARG415	Hydrophobic	5
	Acacetin - Pinocembrin	SER363, ASN382, VAL512, VAL606, GLY367, GLY464	Hydrogen bond	6
		TYR334, TYR572, PHE577, ALA366, ARG415, ALA556	Hydrophobic	6

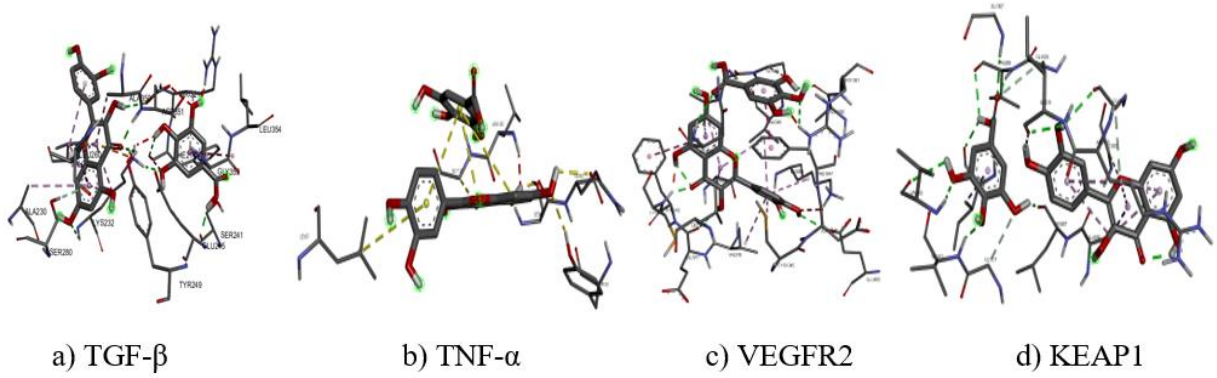

Figure 3 Visualization of the interaction between the receptor and the gallate-quercetin ligand combination at the active sites of TGF-β, TNF-α, VEGFR2, and KEAP1

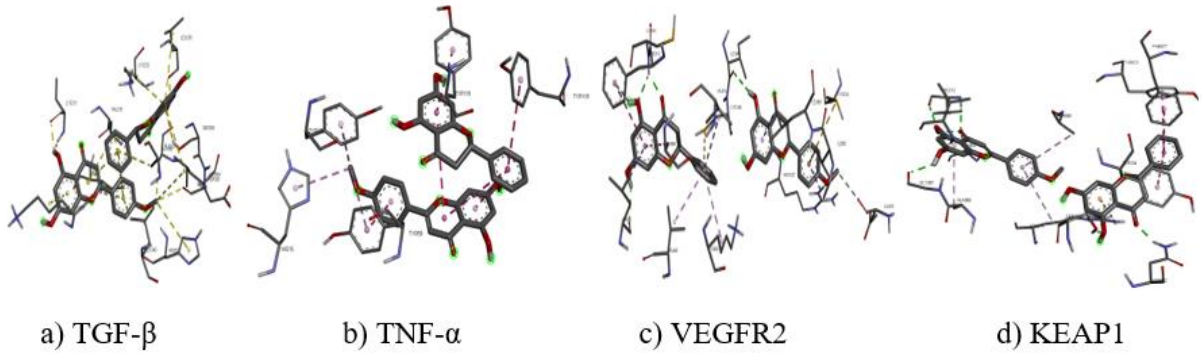

Figure 4 Visualization of the interaction between the receptor and the acacetin-pinocembrin ligand combination at the active sites of TGF-β, TNF-α, VEGFR2, and KEAP1

Fig. 5 and 6 present structural superimposition between the multi-ligand combinations (gallic acid–quercetin and acacetin–pinocembrin) and the native ligands at the binding pockets of TGF-β, TNF-α, VEGFR2, and KEAP1. The visual overlap demonstrates that both compound pairs align closely with the native ligand poses, occupying conserved regions of the active sites. This spatial congruence strongly supports the binding pose reliability of the multi-ligand docking simulations and reinforces their potential to effectively compete with native ligands. In particular, the gallic acid–quercetin combination mimicked native occupancy in the KEAP1 and VEGFR2 receptors, suggesting potential interference with oxidative and angiogenic signaling, respectively.

The overlays also confirm that the tested ligand pairs do not deviate significantly from catalytically relevant regions, minimizing the likelihood of steric hindrance or misalignment. This outcome is important in multi-ligand docking, where inappropriate conformational overlap can artificially inflate docking scores without biological plausibility. According to recent studies, accurate pose overlap within 1to 2 Å of native RMSD is often a more reliable predictor of biological activity than docking score alone [39, 40].

Furthermore, the figures illustrate that the multi-ligand systems enhance binding surface coverage and may engage secondary binding pockets or peripheral residues beyond the reach of single ligands. This broader contact interface aligns with the principles of polypharmacology, wherein multitarget ligands can simultaneously modulate parallel pathways or receptor domains [41]. However, while the spatial alignment strengthens confidence in the docking setup, it remains a static representation. Dynamic behavior and energetic favorability require further validation using molecular dynamics or co-crystallization techniques in future studies.

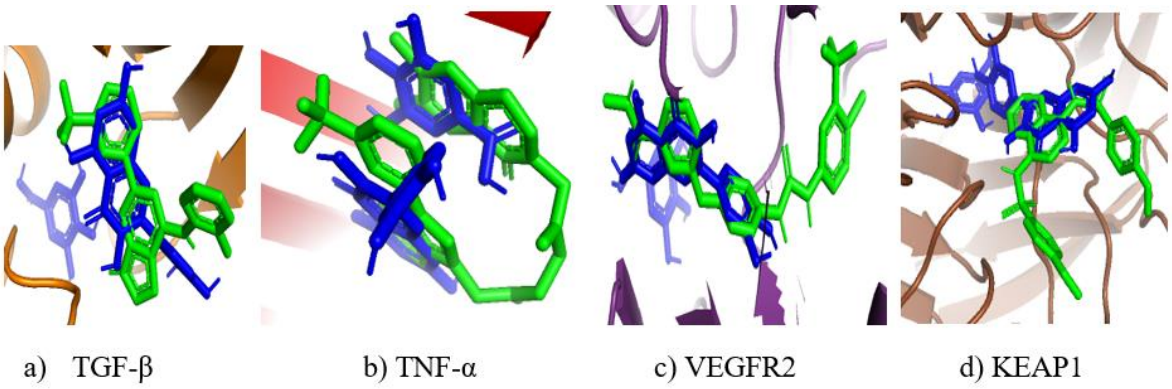

Figure 5 Superimposition of native ligand and gallic acid-quercetin shows the matching of binding sites on all protein targets (green: native ligand, pink: quercetin-error test ligand)

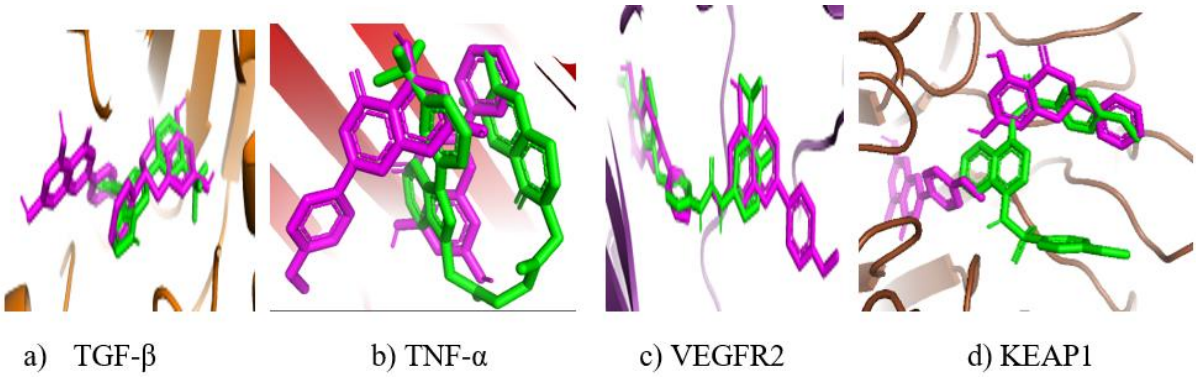

Figure 6 Superimposition of native ligand and multiligand of acacetin-pinocembrin shows optimal overlap at the receptor active site (green: native ligand, pink: test ligand acecatin-pinocembrin)

However, while the interaction profiles are promising, it is important to acknowledge that static docking analyses may overestimate binding stability in the absence of solvation dynamics or receptor flexibility. Thus, the biological relevance of these interaction networks should be validated in future in vitro and in vivo studies, or through dynamic simulations such as MD and MM/PBSA analyses [42]. Further *in vitro* and *in vivo* tests such as observation of cell migration, target protein expression and excisional wound models in animals should be conducted to ensure the effectiveness of this compound in therapeutic topical formulations. This study also marks an innovative molecular-based approach from marine biosources, especially *Spirulina platensis*, in the development of high-potential, efficient and sustainable multifunctional wound healing agents.

4 Conclusion

This study shows that bioactive compounds from *Spirulina platensis*, especially the combination of flavonoids such as gallate-quercetin and acacetin-pinocembrin, have high potential as therapeutic agents for chronic wounds through an *in silico* approach. By targeting four key proteins in the wound healing process (TGF-β, TNF-α, VEGFR2, and KEAP1), the results of molecular docking show that both combinations of these compounds have a stronger binding affinity compared to native ligands. This affinity is supported by the number and strength of hydrogen bonds and high hydrophobic interactions, which indicate the stability of the ligand-receptor complex formed. The combination of gallate-quercetin and acacetin-pinocembrin is able to suppress the inflammatory pathway (TGF-β, TNF-α), modulate angiogenesis (VEGFR2), and activate the antioxidant pathway through inhibition of KEAP1. These synergistic and multitarget effects strengthen the potential of Spirulina as an effective, natural, and sustainable biopharmaceutical candidate in the treatment of chronic wounds. However, these findings are still predictive and require further validation through in vitro and in vivo tests to ensure their biological efficacy and clinical translation. This study

emphasizes the importance of utilizing in silico approaches in natural compound-based drug discovery and paves the way for the development of novel microalgae-based topical formulations for chronic wound therapy.

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