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Research Paper

IN SILICO STUDY OF JATI (*Tectona grandis*) LEAF CONSTITUENTS AS TRADITIONAL WOUND CARE

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ARTICLE HIGLIGHTS

- *T. grandis* leaf methanolic extract contains 82 compounds, with 17 being predominant.
- Epigallocatechin 3-O cinnamate, epigallocatechin 3-O-p-coumarate, and tectograndinol predicted as the potential bioactive compounds in wound healing
- Molecular docking suggests inhibitory activities against NF-κB, MMP-2, and MMP-9, and stimulatory activity against EGFR-1 that comparable to commercial drugs.

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ABSTRACT

Wound-healing process involves a physiological cascade to restore skin integrity, which includes inflammatory response, cell proliferation, and tissue reconstruction. Prolonged inflammation in wound-healing process may lead to a chronic wound stage. Proper wound care is needed to prevent wound-caused mortality. Several studies showed the potential of *T. grandis* leaf in wound-healing process. However, the bioactive compounds and the molecular mechanism of *T. grandis* leaf remains unknown. This study aimed to identify bioactive compounds and biological activity contained in *T. grandis* leaf extract as well as analyze its molecular mechanism in wound-healing process by conducting in silico study using NF-κB, MMP-2, MMP-9, and EGFR-1. Bioactive compounds of *T. grandis* leaf extract were identified with LC-MS. Three potential compounds, epigallocatechin 3-O cinnamate, epigallocatechin 3-O-p-coumarate, and tectograndinol, were selected based on the Pa score screening with the PASS prediction. Drug-likeness and pharmacokinetics properties of the selected compounds were virtually identified by SwissADME and Protox. The interactions of *T. grandis* bioactive compounds toward NF-κB, MMP-2, MMP-9, and EGFR-1 were compared to those of curcumin, phenytoin, and nitrofurazone as control. Molecular docking to identify the protein-ligand interaction was performed by Autodock Vina integrated in PyRx v.0.8. Among 82 bioactive compounds detected in the LC-MS analysis, epigallocatechin 3-O-cinnamate, epigallocatechin 3-O-p coumarate, and tectograndinol exhibited anti-inflammatory, antioxidant, free radical scavenger, and MMP-9 inhibitor activities. According to Lipinski's rule of five, bioactive compounds are possible to be administered as medication. Molecular docking showed that bioactive compounds potentially bound to the active sites of NF-κB, MMP-2, and MMP-9, resulting in proteins inhibition. This study suggested that the wound-healing mechanism of *T. grandis* bioactive compounds were driven by EGFR-1 stimulation indicated by the ability of bioactive compounds to interact with EGFR-1 in similar manner to those of nitrofurazone. We concluded that bioactive compounds of *T. grandis* leaf extract has significant potential to be used as traditional wound treatment and therapy. These compounds demonstrated wound-healing activity in silico by interacting with the key molecular targets, including NF-κB, MMP-2, MMP-9, and EGFR-1.

Keywords: bioactive, EGFR, MMP, Tectona grandis, wound

INTRODUCTION

Skin is the largest barrier of human's body, which provides protection, homeostasis, signal transduction, and excretion. Wound, defined as skin damage, caused by external or internal diseases is threatening and needs proper cure (Tottoli *et al.* 2020). The repairing process included hemostasis, inflammation, proliferation, and remodeling that requires coordination and interaction of various cells, extracellular matrix (ECM), pro-inflammatory mediators, and growth factor (Tyavambiza *et al.* 2022). Though skin has self-recovery ability, various factors delay the wound healing process. Prolonged inflammation might contribute to the development of chronic wounds (Singh *et al.* 2016).

Angiogenesis is a process that promotes the development of chronic wounds which occur immediately after the injury. The mechanism involves matrix metalloproteinases (MMPs) which play major roles in each stage of wound-healing process, such as wound matrix modification, cell migration, angiogenesis, and tissue remodeling. Two types of MMPs, MMP-2 and MMP-9, play a major role in angiogenesis regulation during wound healing via proangiogenic cytokines activation and antiangiogenic peptides generation (Reiss *et al.* 2010; Caley *et al.* 2015; Bergant Suhodolčan *et al.* 2021). Expressions of MMP-2 and MMP-9 are naturally beneficial during the wound-healing process. However, the imbalance level of MMP-2 and MMP-9 may lead to chronic wounds. The abnormal increased level of MMP-9 has been observed in the chronic wounds (Reiss *et al.* 2010; Cabral-Pacheco *et al.* 2020). Elevated MMP-9 expression in the chronic wounds is associated with MMP-2, due to the function of MMP-2 in stimulating MMP-9 activation (Ratzinger *et al.* 2002). The expressions MMP-2 and MMP-9 are often detected in cardiovascular disease, diabetes mellitus, colorectal cancer tissue, and neurodegenerative disease (Groblewska *et al.* 2014; Cabral-Pacheco *et al.* 2020).

Acceleration of wound-healing process is also regulated by the re-epithelialization which occurred during the migration of keratinocytes. Epidermal growth factor receptor (EGFR) is a protein that has been involved in the process of re-epithelization, including cell migration and proliferation (Repertinger *et al.* 2004). Previous findings reported that the increased EGFR expression accelerates wound re-epithelialization on the incisional wound (Nanney *et al.* 2000). The skin of EGFR wild-type mice (EGFR +/+) exhibited smaller erythema at the wound margin compared to EGFR-null mice (EGFR -/-). Five days after the injury, the wound appearance of EGFR-null mice was visibly similar to that of the EGFR wild-type mice (Repertinger *et al.* 2004). EGFR also stimulates angiogenesis in murine skin carcinomas (Casanova *et al.* 2002) which is essential in the early stage of wound healing (Caley *et al.* 2015; Shanthi Kumari *et al.* 2020).

It is important to achieve effective, safe, less cost treatment that provides long-term relief for wounds (Tottoli *et al.* 2020). Natural products are the source to develop traditional and synthetic herbal treatment and therapy with several advantages, such as being readily available in nature, less side effects, and efficiency. Some medicinal plants have been implemented for wound care for several decades (Varma & Giri 2013). *Tectona grandis*, or teak, or jati in Indonesia has been known for its medicinal properties, majorly antioxidant, anti-inflammatory, antibacterial, anti-tumor, and antidiabetic (Varma & Giri 2013; Arief *et al.* 2014; Asdaq *et al.* 2022). Previously, *T. grandis* leaf extract effectively reduced the wound area in the incision and excision wound. It has been reported that the wound area is significantly reduced by treating the wound with 5% and 10% ointment of *T. grandis* leaf extract (Varma & Giri 2013). The anti-inflammatory, antioxidant, immunomodulatory, and antibacterial potential of *T. grandis* leaf are associated in the wound-healing process (Mikhal'chik *et al.* 2006; Dégbé *et al.* 2018; Comino-Sanz *et al.* 2021; Han *et al.* 2023).

Post-surgical wounds and diabetic wounds are more likely to drive a significant impact on a patient's health. Poor healthcare to the patient's wound may develop into chronic wounds. Without proper treatment and therapy, wounds could contribute to mortality (Akiki *et al.* 2021). Thus, treatment of wounds is crucial to reduce the risk of chronic wound development and mortality. Previous in vivo studies reported extensive exploration on the potential of *T. grandis* leaf extract in wound-healing process even though the mechanism is still questionable. This study aimed to provide scientific proof about *T. grandis* potent biological activities, focusing on wound-healing activity. The study was performed by identifying *T. grandis* bioactive compounds, also predicted the biological activity and molecular mechanism in wound-healing process using NF-κB, EGFR1, MMP-2, and MMP-9.

MATERIALS AND METHODS

Preparation of *Tectona grandis* Leaf Extract

T. grandis leaf were obtained from Hutan Jati Gunung Wilis, Madiun. The leaves were identified at the Taxonomy Laboratory of the Universitas PGRI Madiun and were assigned the identification number: 0023/Taxo-Plant/Biology/IV/2021.

The leaves were prepared by drying and grinding into powder, then sieved using 60 mesh size. The processed leaf powder was weighed in 0.5 to 2 g. Leaf powder was extracted with 95% methanol with sample and methanol ratio of 1 : 5 g/mL (Onivogui *et al.* 2015). The powder was macerated with the solution and filtered with Whatman grade 1 which was placed a vacuum filter. The filtrate was evaporated with rotary evaporator to separate the methanol from the extract.

The extract obtained was prepared for analysis with Liquid Chromatography-Mass Spectrometry (LC-MS). Leaf extract was diluted by methanol homogenization until the concentration reached approximately 100 ppm. Sample was centrifuged at 8,000 rpm for 10 minutes to remove any insoluble fine solid particles that could have remained in the extract, thus preventing potential clogging of the analytical column. Two milliliters of supernatant was collected, then 3 mL of acidified acetonitrile was added to the supernatant. The extract was separated by centrifugation at 8,000 rpm for 30 seconds to obtain clear supernatant containing purified sample.

Screening of Bioactive Compounds in *Tectona grandis* Leaf Methanolic Extract

The extract of *T. grandis* leaf was purified using solid phase extraction (SPE) to remove interfering compounds according to the procedure of Fernand *et al.* (2009). The sample was loaded to the C18 Sep-Pak cartridge column (1 mL, 100 mg) which had previously been conditioned with 1 mL mixture of acetonitrile : water with ratio of 80 : 20 to activate the stationary phase.

Approximately 0.5 mL of the liquid leaf extract was then loaded onto the conditioned column. The column was washed with a series of solutions to selectively elute different compound classes. Specifically, 1 mL of water was added to remove highly polar impurities. Subsequently, 0.5 mL of acetonitrile : water (80 : 20 ratio) was added to elute semi-polar compounds. Finally, 0.5 mL of acetonitrile was used to elute the most non-polar compounds of interest. The final purified solution was filtered using a 0.45 µm cellulose acetate membrane filter and degassed before injection. The sample was analyzed using a Shimadzu LCMS-8040 Liquid Chromatography-Mass Spectrometry system. The injection volume was 1 µL.

Chromatographic separation was performed on a Shimadzu Shim-pack FC-ODS column (2 mm x 150 mm, 3 µm). The mobile phase consisted of a single solvent system in isocratic mode. The mobile phase was methanol : water (90 : 10 ratio), delivered at a constant flow rate of 0.5 mL/minute. The total run time was 80 minutes. The mass spectrometer was operated in positive ion mode. The ion source was Electrospray Ionization (ESI), interface voltage +4.5 kV, desolvation line (DL) temperature of 250 °C, heat block temperature of 400 °C, drying gas flow of 15 L/minute, nebulizing gas flow of 3 L/minute, and scan mode with full scan (m/z range 100–1000).

Bioactivity Prediction of *Tectona grandis* Bioactive Compounds

Seventeen most abundant bioactive compounds of *T. grandis* leaf extract were identified for their potency, which we selected for further analyses (Table 1). The canonical SMILES of 17 bioactive compounds were obtained from PubChem and loaded to PASS prediction for bioactivity prediction (Filimonov *et al.* 2014). Each bioactive compound was later identified for the potency as antioxidant, free radical scavenger, MMP expression inhibitor, and anti-inflammatory. Bioactive compounds with Pa score ≥ 0.7 were chosen for further analysis, as they indicate a high similarity to compounds in the database that have been proven for treatment and therapy.

Prediction of Drug-likeness and Toxicity of Bioactive Compounds

Three bioactive compounds selected for bioactivity predictions were continued for identification and prediction of its drug-likeness and pharmacokinetics (Banerjee *et al.* 2024). The canonical SMILES of three bioactive compounds were loaded to SwissADME (http://www.swissadme.ch/index.php) and Protox tool (https://tox.charite.de/protox3/index.php).

Table 1 Dominant bioactive compounds of *Tectona grandis* leaf extract

Retention time	Composition (%)	Bioactive compound	Pubchem ID
23.293	2.48141	Epigallocatechin-3-O-p coumarate	CID6474788
23.705	2.17803	Epigallocatechin gallate	CID65064
33.505	2.15389	Procyanidin B5	CID124017
22.176	2.13232	6C-glucopyranosylepicatechin	CID131752183
33.434	2.09243	Proanthocyanidin A-2	CID124025
33.496	1.98748	Procyanidin B1	CID11250133
11.566	1.84348	Isoobtusilactone A	CID6442493
3.042	1.80174	Gallic acid	CID370
11.958	1.77578	Tectograndinol	C_ID C00022743 (from knapsack)
21.584	1.7546	Epigallocatechin-3-O cinnamate	CID21629801
19.211	1.73830	Epiafzelechin-3-O-gallate	CID467295
33.498	1.71418	Procyanidin B2	CID124017
10.322	1.70537	Kaempferol	CID5280863
33.429	1.58901	Procyanidin A1	CID5089889
11.562	1.43720	Isolinderanolide B	SID274339182
12.421	1.43717	Chlorogenic acid	CID1794427
11.427	1.42081	Quercetin	CID5280343

Molecular Docking

Selected bioactive compounds of *Tectona grandis* leaf extract were further analyzed for the interactions toward proteins involved in wound-healing process. The 3D structures of selected bioactive compounds, i.e., epigallocathecin 3-O-p coumarate (CID6474788) and epigallocatechin 3-O-cinnamate (CID21629801), were downloaded from PubChem database. The 3D structure of tectograndinol was obtained from the canonical SMILES structure provided by the Knapsack family C_ID C00022743 and loaded to OpenBabel to construct its 3D chemical structure, later converted into SDF format. Curcumin (CID969516), nitrofurazone (CID5447130) and phenytoin (CID1775), as a control, were obtained from PubChem. All ligands, including bioactive compounds and control, were prepared using PyRx 0.8 by minimizing the energy and converting into PDB format.

The 3D structure of EGFR-1 (3POZ), MMP9 (1L6J), MMP2 (7XGJ), and NF-κB (1KN) were downloaded from PDB (Liu *et al.* 2021; Al Mousa *et al.* 2024). Proteins were prepared by removing water molecules and the natural ligands with Discovery Studio 4.1. Ligands were prepared by minimizing the energy and converting to PDB format in PyRx version 0.8. Docking was performed by AutoDock Vina integrated in PyRx 0.8. Bioactive compounds were interacted to EGFR-1 (x = 19.5327, y = 24.7294, z = 15.1872), MMP-9 (x = 39.3102138564, y = 35.2175327806, z = 34.6209), MMP-2 (x = 20.3416, y = -0.3234, z = 20.0629), and NF-κB (x = 38.4173, y = 25.9442, z = 28.1869) in the specific grid box optimized by the software and exhaustiveness 50. Interactions between ligands and proteins were visualized by Discovery Studio 4.1.

RESULTS AND DISCUSSION

Prediction on Biological Activity of *T. grandis* Leaf Extract

Methanolic extracts of *T. grandis* leaf contained 82 bioactive compounds based on the LC-MS analysis (Fig. 1). Bioactive compounds with high peak in the LC-MS result as well as longer retention time were categorized as the abundant bioactive compound and predicted as the dominants. However, some bioactive compounds may elute later due to the strong interaction between analyte and the stationary phase, showing the late in retention time (Katajamaa & Oresic 2005).

Our study found that the most abundant bioactive compounds were flavonoids group, followed by proanthocyanidin, phenolic acids, diterpenoids, and lignans. Other studies showed that the *T. grandis* leaf extract contains various bioactive compounds, such as flavonoids, tannins, alkaloids, anthraquinones, anthocyanins, naphthoquinone, and cyanidine (Arief *et al.* 2014; Murukan & Murugan 2018). Bioactive compounds exhibit various biological activities,

such as anti-inflammatory, antibacterial, and antioxidants causes the plant to be a traditional agent for treating diabetes, cancer, malaria, and skin diseases (Rajuri *et al.* 2010; Suryanti *et al.* 2020). Phenolic contents are the major constituent of *T. grandis* leaf extract (Budianto *et al.* 2023). Phenolic and anthocyanins of *T. grandis* leaf methanol extract exerts strong antioxidant activity (Suryanti *et al.* 2020). The antioxidant activity of *T. grandis* allowed the acceleration of the wound-healing process by reducing the oxidative stress and performing anti-inflammatory activity (Ponugoti *et al.* 2013; Comino-Sanz *et al.* 2021).

Seventeen bioactive compounds were screened for biological activity-related to wound-healing mechanisms, which are on anti-inflammatory activity, free radical scavenger, antioxidant activity, and MMP inhibitory activity (Table 2).

According to the screening, bioactive compounds of *T. grandis* leaf extract mostly exhibited antioxidant and anticarcinogenic activities. Tectograndinol and epigallocatechin-3-O-p coumarate showed exceptional activities as anti-inflammatory and MMP-9 inhibitor, respectively. Epigallocatechin 3-O-p-coumarate and epigallocatechin-3-O-cinnamate also showed antioxidant potential, as indicated by their high predicted Pa scores for free radical scavenging and antioxidant activities. Furthermore, these bioactive compounds exhibited the highest Pa scores for all biological activities included in the prediction. Therefore, epigallocathecin 3-O-cinnamate, epigallocathecin 3-O-p coumarate, and tectograndinol were selected for further analysis.

The anti-inflammatory activity of epigallocathecin 3-O-cinnamate and epigallocathecin 3-O-p coumarate may be performed by flavonoids with two connecting benzene rings (A and B) via oxygen-containing heterocycle (C), also with the A ring of glycosyl mode (Maleki *et al.* 2019; Han *et al.* 2023). Tectograndinol which appeared with the highest anti-inflammatory activity, categorized as diterpenoid group. Diterpenoids are the promising class of secondary metabolites with presenting high anti-inflammatory capacity (González *et al.* 2015).

Commercial medicinal plant extracts containing high diterpenoids showed direct anti-inflammatory activity by inhibiting NF-κB, which effectively reduced pain and symptoms of rheumatoid arthritis (González *et al.* 2015; Lv *et al.* 2015). Seven diterpenoid glucosides from fruit extracts presents anti-inflammatory activity against LPS-induced NO production in RAW 264.7 (Liu *et al.* 2023).

Previous study revealed the decreased pro-inflammatory mediators, TNF-α and interleukins (IL-1β, IL-6), on the macrophage-stimulated LPS following treatment with *T. grandis* leaf extract (Han *et al.* 2023). The complexity of phytochemical constituents from *T. grandis* also allowed physiological mechanisms. Additionally, bioactive compounds from the extract could modulate the immune response to impede the parasitic effect on antigen presenting cells by increasing the cytokine production (Dégbé *et al.* 2018).

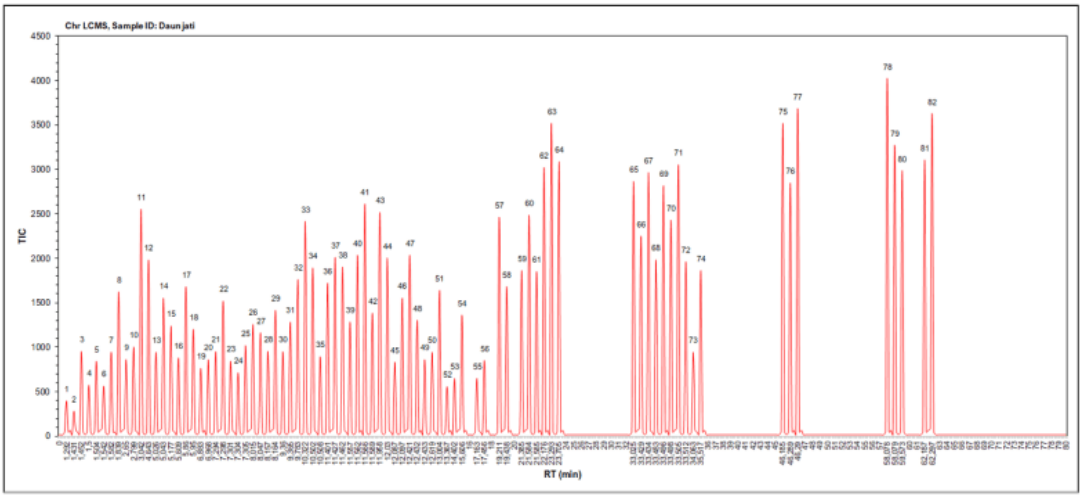

Figure 1 Bioactive compounds of *Tectona grandis* leaf extract analyzed by liquid chromatography-mass spectrometry (LC-MS) spectra

Table 2 Biological activity prediction of *Tectona grandis* leaf extract

Bioactive compound		Free radical scavenger	Antioxidant	MMP9 expression inhibitor	Anticarcinogenic	Anti-inflammatory
Epigallocatechin 3-O-p-coumarate	Pa	**0.96**	**0.87**	0.706	**0.897**	
	Pi	0.001	0.003	0.006	0.003	
Epigallocatechin gallate	Pa	0.934	0.814		0.841	
	Pi	0.001	0.003		0.004	
Procyanidin B5	Pa	0.856	0.787		0.848	
	Pi	0.002	0.004		0.004	
(-)-Epicatechin 6-C-glucoside	Pa	0.894	0.831			
	Pi	0.002	0.003			
Proanthocyanidin A-2	Pa	0.742	0.704		0.713	
	Pi	0.003	0.004		0.008	
Procyanidin B1	Pa	0.798	0.803		0.757	
	Pi	0.003	0.003		0.007	
Isoobtusilactone A	Pa					
	Pi					
Gallic acid	Pa					
	Pi					
Tectograndinol	Pa					**0.746**
	Pi					0.011
Epigallocatechin-3-O-cinnamate	Pa	**0.953**	**0.863**		**0.891**	
	Pi	0.001	0.003		0.003	
Epiafzelechin-3-O-gallate	Pa	0.933	0.81		0.844	
	Pi	0.001	0.003		0.004	
Procyanidin B2	Pa	0.798	0.803		0.757	
	Pi	0.003	0.003		0.007	
Kaempferol	Pa	0.771	0.856	0.738	0.715	
	Pi	0.003	0.003	0.005	0.008	
Procyanidin A1	Pa	0.742	0.704		0.713	
	Pi	0.003	0.004		0.008	
Isolinderanolide B	Pa					
	Pi					
Chlorogenic acid	Pa	0.856	0.785		0.846	
	Pi	0.002	0.004		0.004	
Quercetin	Pa	0.811	0.872	0.734	0.757	
	Pi	0.003	0.003	0.005	0.007	

Drug-likeness and Pharmacokinetics Prediction of *Tectona grandis* Bioactive Compounds

Prior to drug development, it is necessary to predict the drug-likeness and pharmacokinetics properties of the bioactive compounds. Drug-likeness properties according to Lipinski's rule of five and pharmacokinetics include absorption, distribution, metabolism, excretion, and toxicity (ADME/T) which contribute to the physiological effect of a compound (Krisnamurti *et al.* 2021; Banerjee *et al.* 2024). According to the ADME/T analysis, epigallocatechin 3-O-p coumarate has 1 violation (Table 3). However, the bioavailability score of bioactive compounds was 0.55, which is still in the range of good bioavailability score. It means the bioactive compounds can be easily absorbed by the body (Martin 2005).

Compared to others, tectograndinol exhibits the highest gastrointestinal absorption and blood brain barrier permeability. Epigallocatechin 3-O-cinnamate, epigallocatechin 3-O-p-coumarate, and tectograndinol shared similar properties in which lipophilicity and water solubility. The bioactive compounds are more likely lipophilic and moderately soluble in water.

The log Kp score is important in the analysis of *T. grandis* bioactive compounds, as wound treatment is mostly used for skin permeability. The log Kp value represents how easy the chemical can penetrate the skin, which indicates the significance of skin absorption. The more negative log Kp value the lower the potency of a bioactive compound to penetrate the skin (Scheler *et al.* 2014). All three bioactive compounds showed negative scores of skin permeation, while tectograndinol showed the less negative score. Virtual prediction indicates tectograndinol as the potent bioactive compound for external application for wound treatment, such as an ointment.

Though bioactive compounds are known for its safety and less adverse effect, it is important to test the toxicity. Toxicity analysis was carried out by predicting LD50 and various toxicity properties in the system. LD50 and class of toxicity represent the amount of a substance that is expected to cause death in 50% of a group of test animals from a single dose. Meanwhile, various toxicity properties specifically predict the types of adverse effects that can emerge in an organism (Oduola *et al.* 2010).

The bioactive compounds tested in this study were categorized in class 4 and class 5. The LD50 were divided into six categories, i.e., class I (LD50 ≤ 5 mg/kg), class II (5 < LD50 ≤ 50 mg/kg), class III (50 < LD50 ≤ 300 mg/kg), class IV (300 < LD50 ≤ 2,000 mg/kg), class V (2,000 < LD50 ≤ 5,000 mg/kg), and class VI (LD50 > 5,000 mg/kg). The higher the class of LD50 the less harmful the bioactive compound to the body (Banerjee *et al.* 2024). Our study showed that tectograndinol possessed less toxicity compared to those of epigallocatechin 3-O-p-coumarate and epigallocatechin 3-O-cinnamate.

Toxicity prediction showed that epigallocathecin 3-O-cinnamate and epigallocatechin 3-O-p-coumarate had the probability of nephrotoxicity and respiratory toxicity of 0.72 and 0.78, respectively, for both compounds. The nephrotoxicity and respiratory toxicity were probably inactive at the probability of 1 – 0.72 and 1 – 0.78, respectively. Moreover, those compounds probably caused immunotoxicity as the score were the highest at 0.9 and 0.94. The probability of compounds to be inactive was less than 10%. Tectograndinol showed the highest toxicity in cardiotoxicity with score of 0.83, while it may inactive with probability of 1 – 0.83 which is score 0.17 (17%). Overall, bioactive compounds of *T. grandis* leaf extract showed its safety by not causing hepatotoxicity, neurotoxicity, carcinogenicity, mutagenicity, and cytotoxicity. The bioactive compounds are also safe for the environment, represented by ecotoxicity, so it could be predicted that the bioactive compounds are ecofriendly as if they were developed as a drug (Gan *et al.* 2024). However, development of epigallocatechin 3-O-cinnamate and epigallocatechin 3-O-p-coumarate as a medication may need further consideration as they might be toxic in nephrons, indicated by the red color in the table. According to ADME/T analysis, tectograndinol is potentially the safest bioactive compound for drug development.

Table 3 Drug-likeness and pharmacokinetics properties of *Tectona grandis* bioactive compounds

	Epigallocathecin 3-O-cinnamate	**Epigallocatechin 3-O-p-coumarate**	**Tectograndinol**
Lipinski	**Yes; 0 violation**	**Yes; 1 violation**	**Yes; 0 violation**
Bioavailability score	0.55	0.55	0.55
Lipophilicity (Po/w)	2.45	2.07	3.4
Water solubility (LogS)	-5.18 (Moderately soluble)	-5.24 (Moderately soluble)	-4.24 (Moderately soluble)
GI absorption	Low	Low	High
BBB permeant	No	No	Yes
P-gp substrate	No	No	No
CYP1A2 inhibitor	No	No	No
CYP2C19	No	No	No
CYP2C9	No	No	No
CYP2D6	No	No	No
CYP3A4	No	No	No
Log Kp (skin permeation)	-7.07 cm/s	-7.42 cm/s	-5.93 cm/s
LD50	1000 mg/kg	1000 mg/kg	5000 mg/kg
Toxicity	4	4	5

	Epigallocathecin 3-O-cinnamate	Epigallocatechin 3-O-p-coumarate	Tectograndinol
Lipinski	**Yes; 0 violation**	**Yes; 1 violation**	**Yes; 0 violation**
Hepatotoxicity	0.68	0.68	0.86
Neurotoxicity	0.85	0.85	0.85
Nephrotoxicity	0.72	0.72	0.74
Respiratory toxicity	0.78	0.78	0.73
Cardiotoxicity	0.87	0.87	0.83
Carcinogenicity	0.52	0.52	0.61
Immunotoxicity	0.90	0.94	0.63
Mutagenicity	0.64	0.64	0.75
Cytotoxicity	0.77	0.77	0.96
BBB-barrier	0.54	0.54	0.61
Ecotoxicity	0.6	0.6	0.54
Clinical toxicity	0.54	0.54	0.68
Nutritional toxicity	0.64	0.64	0.74

Notes: Less active | Active | Less inactive | Inactive

Interactions of *Tectona grandis* Bioactive Compounds to Wound Healing-Related Protein

Identification of wound-healing mechanism was performed by in silico study using key wound healing proteins, NF-κB, MMP-9, MMP-2, and EGFR-1. The potency of three selected *T. grandis* bioactive compounds on wound-healing-related protein were compared to curcumin, nitrofurazone, and phenytoin as control.

Curcumin, a polyphenol derived from Curcuma longa, is widely known as potential anti-inflammatory and antioxidant agent. Study demonstrated the activity of curcumin in reducing the healing time on excisional wound in rats, which mostly plays in the proliferative phase of wound-healing process (Patenall *et al.* 2024).

Nitrofurazone is an antibacterial agent that has been acknowledged for its potency in wound care. Study revealed the activity of nitrofurazone in promoting tissue granulation in the wound, thus leads to wound-healing process (Erdur *et al.* 2008).

Phenytoin or diphenylhydantoin was firstly established as convulsive disorders treatments. It has an effect on connective tissue and is potentially used in wound care. Study demonstrated the wound-healing process in rat burn skin wound model following phenytoin administration. Phenytoin aids wound-healing process by developing vascularized and granulation tissue, also synthesizing collagen by re-epithelization (Patenall *et al.* 2024).

The activity of bioactive compounds toward NF-κB and MMPs were compared with phenytoin, while nitrofurazone was used to compare the activity of bioactive compounds toward EGFR-1. The activity of *T. grandis* bioactive compounds against NF-κB were shown in Figure 2.

NF-κB is an essential transcriptional factor in wound-healing process, which plays major role in cell migration in inflammatory phase and proliferation in proliferative phase. The activation of NF-κB affects the cytokines and growth factor secretion, cell proliferation, also MMPs expression (Yadav *et al.* 2024). Our study revealed that bioactive compounds from *T. grandis* leaf extract performed inhibition toward NF-κB in the similar inhibitory sites as phenytoin (Table 4).

Phenytoin posed binding with Glu49, Arg50, Glu222, Gln241, and Gly259 of NF-κB (-8 kcal/mol) in which those amino acid residues indicated as the key amino acid residues of NF-κB inhibition. Epigallocatechin 3-O-cinnamate performed inhibition via Glu222 and Gln241 (-9 kcal/mol), epigallocatechin 3-O-p-coumarate via Arg50 (-9.8 kcal/mol), while tectograndinol via Glu49, Gln241, and Gly259 (-8 kcal/mol). It could be predicted that *T. grandis* compounds performed wound-healing process in similar manner as the commercial wound-healing medication.

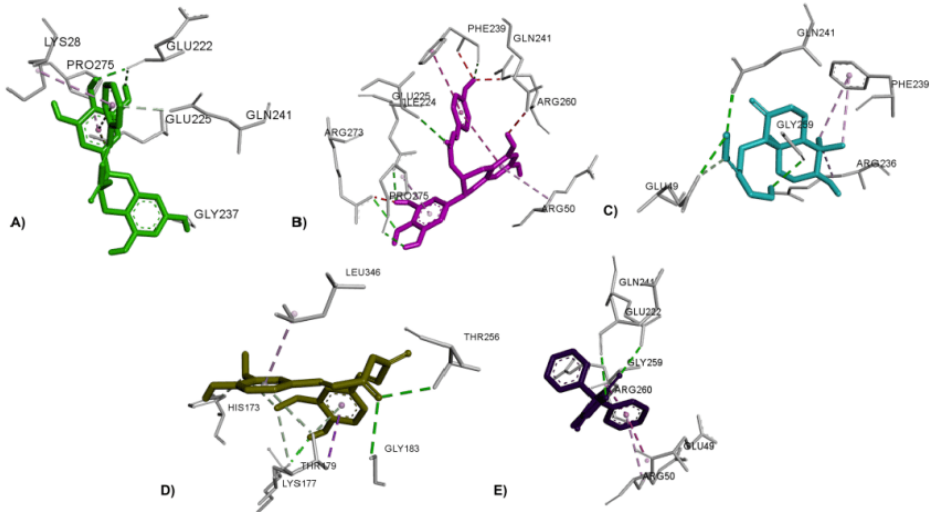

Figure 2 Interaction of with ligands

Notes: A = epigallocathecin 3-O-cinnamate; B = epigallocatechin 3-O-p-coumarate; C = tectograndinol; D = curcumin; E = phenytoin. Bioactive compounds from *T. grandis* leaf extract potentially formed binding with the similar amino acid residues as phenytoin.

Table 4 Interactions of *Tectona grandis* bioactive compounds toward NF-κB

	Ligand	Name	Category	Type	Binding energy (kcal/mol)
NF-κB	Epigallocatechin 3-O-cinnamate	Glu222, Gly237	Hydrogen Bond	Conventional Hydrogen Bond	-9
		Gln241	Hydrogen Bond	Pi-Donor Hydrogen Bond	
		Glu225	Electrostatic	Pi-Anion	
		Pro275, Lys28	Hydrophobic	Pi-Alkyl	
	Epigallocatechin 3-O-p-coumarate	Phe239, Glu225, Pro275, Ile224, Arg273	Hydrogen Bond	Conventional Hydrogen Bond	-9.8
		Phe239	Hydrophobic	Pi-Pi T-shaped	
		Arg50, Pro275	Hydrophobic	Pi-Alkyl	
	Tectograndinol	Glu49. Gly259, Gln241	Hydrogen Bond	Conventional Hydrogen Bond	-7.2
		Glu49	Hydrogen Bond	Carbon Hydrogen Bond	
		Arg236	Hydrophobic	Alkyl	
		Phe239	Hydrophobic	Pi-Alkyl	
	Curcumin	Lys177, Thr256, Gly183	Hydrogen Bond	Conventional Hydrogen Bond	-8
		Lys177, His173	Hydrogen Bond	Carbon Hydrogen Bond	
		Thr179	Hydrogen Bond	Pi-Donor Hydrogen Bond	
		Thr179	Hydrophobic	Pi-Sigma	
		Leu346	Hydrophobic	Pi-Alkyl	
	Phenytoin	Glu222, Gln241	Hydrogen Bond	Conventional Hydrogen Bond	-8
		Glu49;Arg50, Gly259;Arg260	Hydrophobic	Amide-Pi Stacked	
		Arg50	Hydrophobic	Pi-Alkyl	
		Asp390	Electrostatic	Pi-Anion	
		Tyr393	Hydrophobic	Pi-Pi Stacked	
		Pro97	Hydrophobic	Pi-Alkyl	

The binding sites of *T. grandis* bioactive compounds toward NF-κB were different from that of curcumin, which suggested that *T. grandis* bioactive compounds act toward NF-κB differently from curcumin, the common nature-based wound-healing agent. The potency of *T. grandis* bioactive compounds to inhibit NF-κB also indicated by lower binding energy than those of curcumin and phenytoin. A low binding energy indicates the efficient ability of ligands to bind strongly to their receptors. A receptor-ligand complex with low binding energy is expected to form a strong and stable complex. In contrast, a higher binding energy suggests a weaker binding interaction, which may negatively affect the ligand's ability to effectively perform its function with the receptor (Hasan *et al.* 2023).

Epigallocatechin 3-O-cinamate, epigallocatechin 3-O-p-coumarate, and tectograndinol formed binding sites toward MMP-2 differently to each other (Fig. 3). In the binding to MMP-2, tectograndinol did not share any identical amino acid residues with the other compounds. However, tectograndinol was an exception to this observation, as it shared two key amino acid residues with the control drug phenytoin, specifically at Pro25 and His98 with binding energy of -6.6 kcal/mol.

A study conducted by Takeuchi *et al.* (2022) revealed that the interaction of an inhibitor with amino acid residues in the catalytic site, including His121, His131, Glu130, Ala88, Phe87, Ala86, Phe5, Tyr74, and Leu82 is crucial for the downregulation of MMP-2 expression. Based on molecular docking study, epigallocathecin 3-O-cinnamate may perform MMP-2 inhibition by binding on MMP-2 catalytic sites Phe87, Ala88, and Tyr74 with strongest binding energy -7.9 kcal/mol (Hasan *et al.* 2023). Epigallocatechin 3-O-p-coumarate moderately acts as MMP-2 inhibitor by binding to Phe5 with -7.4 kcal/mol.

Our study showed that curcumin possessed the potential activity to bind with MMP-2 indicated by the highest binding energy score (-9.5 kcal/mol), while it did not interact with MMP-2 catalytic sites. This molecular docking study revealed that *T. grandis* bioactive compounds are more potent to bind with MMP-2, compared to binding with curcumin and phenytoin, as the bioactive compounds bind with catalytic sites with lower binding energy than phenytoin. It suggested that *T. grandis* bioactive compounds may inhibit collagen, fibronectin, laminin, and elastin degradation (Wolosowicz *et al.* 2024).

Our study revealed that bioactive compounds of *T. grandis* leaf extract showed less inhibitory activity on MMP-9 (Fig. 4). The results suggested that the binding mechanism of *T. grandis* bioactive compounds to MMP-9 is distinct from that of curcumin or phenytoin, as evidenced by the lack of shared amino acid residues at the binding site (Table 5).

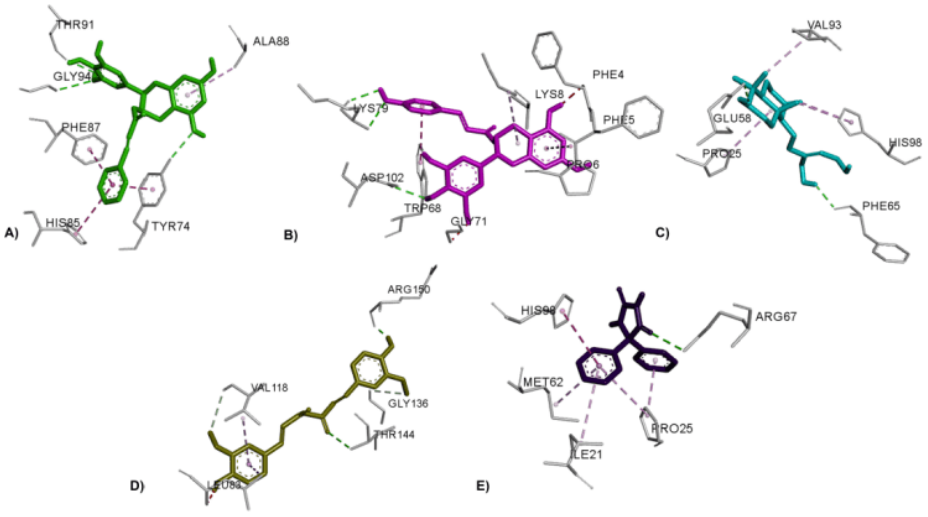

Figure 3 Interaction of MMP-2 with ligands

Notes: A = epigallocathecin 3-O-cinnamate; B = epigallocatechin 3-O-p-coumarate; C = tectograndinol; D = curcumin; E = phenytoin. All compounds posed different binding sites, suggesting the difference of mechanism of action to MMP-2.

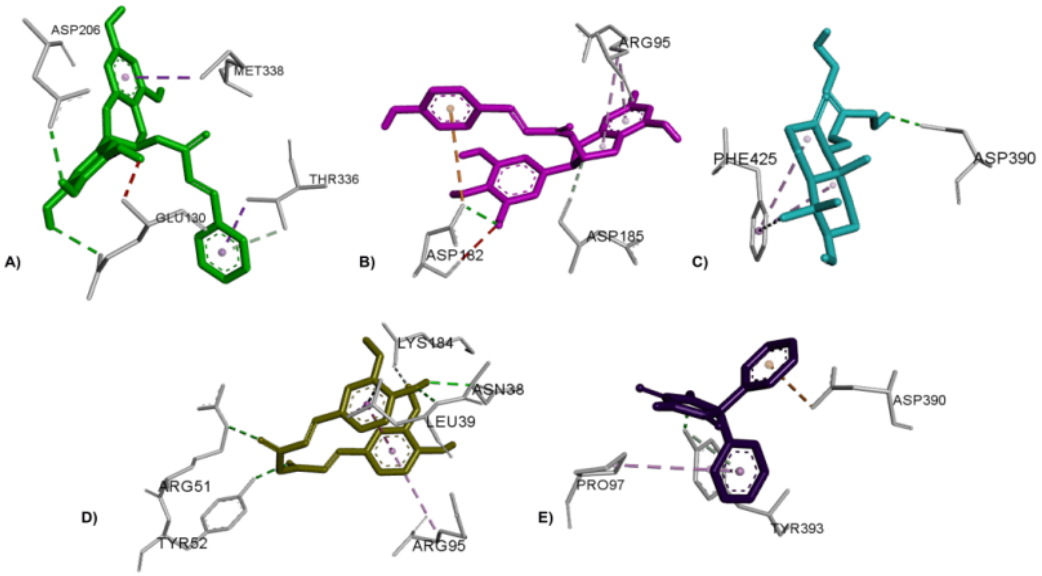

Figure 4 Interaction of MMP-9 with ligands

Notes: A = epigallocathecin 3-O-cinnamate; B = epigallocatechin 3-O-p-coumarate; C = tectograndinol; D = curcumin; E = phenytoin. Tectograndinol appeared to have similar binding site as phenytoin.

Table 5 Interactions of *Tectona grandis* bioactive compounds toward MMPs

	Ligand	Name	Category	Type	Binding energy (kcal/mol)
MMP-2	Epigallocathecin 3-O-cinnamate	Thr91, Tyr74, Gly94	Hydrogen Bond	Conventional Hydrogen Bond	-7.9
		Tyr74	Hydrophobic	Pi-Pi Stacked	
		Phe87, His85	Hydrophobic	Pi-Pi T-shaped	
		Ala88	Hydrophobic	Pi-Alkyl	
	Epigallocatechin 3-O-p-coumarate	Lys79, Asp102	Hydrogen Bond	Conventional Hydrogen Bond	-7.4
		Trp68	Hydrophobic	Pi-Pi T-shaped	
		Phe5;Pro6	Hydrophobic	Amide-Pi Stacked	
		Lys8	Hydrophobic	Alkyl	
		Gly71, Phe4	Unfavorable	Unfavorable Donor-Donor	
	Tectograndinol	Phe65, Glu58	Hydrogen Bond	Conventional Hydrogen Bond	-6.6
		Pro25, Val93	Hydrophobic	Alkyl	
		His98	Hydrophobic	Pi-Alkyl	
	Curcumin	Arg150, Thr144	Hydrogen Bond	Conventional Hydrogen Bond	-9.5
		Gly136, Val118	Hydrogen Bond	Carbon Hydrogen Bond	
		Leu83	Hydrophobic	Pi-Sigma	
		Val118	Hydrophobic	Pi-Alkyl	
	Phenytoin	Arg67	Hydrogen Bond	Conventional Hydrogen Bond	-6.9
		His98	Hydrophobic	Pi-Pi T-shaped	
		Pro25, Ile21, Met62	Hydrophobic	Pi-Alkyl	

	Ligand	Name	Category	Type	Binding energy (kcal/mol)
MMP-9	Epigallocathecin 3-O-cinnamate	Asp206, Glu130	Hydrogen Bond	Conventional Hydrogen Bond	-7.4
		Thr336	Hydrogen Bond	Pi-Donor Hydrogen Bond	
		Thr336, Met338	Hydrophobic	Pi-Sigma	
	Epigallocatechin 3-O-p-coumarate	Asp182	Hydrogen Bond	Conventional Hydrogen Bond	-7.9
		Asp185	Hydrogen Bond	Carbon Hydrogen Bond	
		Asp182	Electrostatic	Pi-Anion	
		Arg95	Hydrophobic	Alkyl; Pi-Alkyl	
	Tectograndinol	Asp390	Hydrogen Bond	Conventional Hydrogen Bond	-5.9
		Phe425	Hydrophobic	Pi-Alkyl	
	Curcumin	Asn38, Leu39, Arg51, Tyr52	Hydrogen Bond	Conventional Hydrogen Bond	-7.4
		Lys184	Hydrogen Bond	Carbon Hydrogen Bond	
		Leu39	Hydrophobic	Pi-Sigma	
		Arg95	Hydrophobic	Pi-Alkyl	
	Phenytoin	Tyr393	Hydrogen Bond	Conventional Hydrogen Bond; Pi-Donor Hydrogen Bond	-6.1
		Asp390	Electrostatic	Pi-Anion	
		Tyr393	Hydrophobic	Pi-Pi Stacked	
		Pro97	Hydrophobic	Pi-Alkyl	

However, tectograndinol was an exception, as it shared the same amino acid residue, Asp390, with the phenytoin. Tectograndinol was suggested to inhibit MMP-9 in the inflammation, as the compound also formed binding with Phe425, which is known as the active site of MMP-9 (Elkins *et al.* 2002). Neither epigallocatechin 3-O-cinnamate nor epigallocatechin 3-O-p-coumarate formed binding interactions with MMP-9 at the same amino acid residues as phenytoin or curcumin, suggesting a different inhibitory mechanism. Bioactive compounds of *T. grandis* actively inhibit MMP-9, as shown by their lower binding energy in the docking study. Epigallocathecin 3-O-p-coumarate performed the strongest binding to MMP-9 (-7.9 kcal/mol). Epigallocathecin 3-O-cinnamate binding to MMP-9 was the second strongest (-7.4 kcal/mol) and tectograndinol binding to MMP-9 was the least strong (-5.9) kcal/mol). The ability of tectograndinol to interact with MMP-9 was relatively lower as compared with control.

Molecular docking result showed the optimum binding of *T. grandis* leaf bioactive compounds toward EGFR-1 (Table 6). *T. grandis* compounds, epigallocathecin 3-O-cinnamate, epigallocathecin 3-O-p-coumarate, and tectograndinol, were specifically bind to EGFR-1 through Val726, Ala743, and Lys745 (Fig. 5).

Moreover, nitrofurazone and curcumin also occupy the same binding pocket as *T. grandis* bioactive compounds, which indicate the potential of *T. grandis* bioactive compounds to promote wound-healing process in a similar manner to those of nitrofurazone and curcumin. Based on the binding energy, epigallocathecin 3-O-cinnamate was the easiest to bind with EGFR-1 (-9.1 kcal/mol). Epigallocathecin 3-O-p-coumarate performed a moderate strength with -8.9 kcal/mol binding affinity. The least binding energy was on tectograndinol with -7.4 kcal/mol.

Binding of *T. grandis* leaf bioactive compounds to the active sites of EGFR-1 may stimulate the activation of EGFR-1 for cell migration and proliferation, leading to re-epithelialization and dermal maturation (Repertinger *et al.* 2004; Shin *et al.* 2022). Based on the virtual prediction, *T. grandis* bioactive compounds have as much potential as nitrofurazone in accelerating the wound-healing process. Treatment with nitrofurazone increased the re-epithelialization process and decreased the number of inflammatory cells. The bactericidal effect of nitrofurazone contributed to the acceleration of the wound-healing process (Karapolat *et al.* 2020).

Interestingly, a previous study reported the antibacterial activity of anthraquinones in *T. grandis* extract. The methanolic and aqueous extract of *T.*

grandis has also demonstrated the inhibitory effect on Gram-positive and Gram-negative bacteria (Irinmwinuwa *et al.* 2023). Treatment of wounds with *T. grandis* leaf extract may have more potency than that of nitrofurazone.

The process of wound healing requires various types of cells and proteins. MMP is one of the proteins regulated during wound-healing process that is activated by NF-κB and upregulated during inflammation stage which mostly driven by reactive oxygen species (ROS) (Caley *et al.* 2015; Kandhwal *et al.* 2022; Yadav *et al.* 2024). The balance level of ROS may stimulate the signaling pathway of NF-κB activation and MMPs upregulation, which play major role during wound-healing process. However, ROS could also stimulate the ECM turnover, causing tissue destruction and interference with the repair process (Hingorani *et al.* 2019). Besides, the prolonged inflammation phase would also delay the wound-healing process. Treatment with *T. grandis* leaf extract with exhibit antioxidant and free radical scavenger activity may balance the ROS level during wound-healing process, leading to the acceleration of wound-healing process (Sun *et al.* 2021).

A molecular docking study revealed the potential of bioactive compounds from *T. grandis* in treating wounds, showing a similar mechanism to the known drugs phenytoin and nitrofurazone. In contrast, the wound-healing mechanism of *T. grandis* leaf compounds may differ slightly from that of curcumin, a natural product. This is supported by the finding that curcumin and the *T. grandis* compounds bind to different sites on the key wound-healing proteins. Furthermore, the study revealed that *T. grandis* leaf bioactive compounds bind to the active sites of NF-κB, MMP-2, MMP-9, and EGFR-1 with high efficiency. Based on virtual prediction, our study suggested the mechanism of *T. grandis* leaf extract in wound-healing process via NF-κB, MMP-2, MMP-9, and EGFR-1 (Fig. 6), however, further investigation through in vivo and in vitro studies is required for definitive confirmation.

Table 6 Interaction of *Tectona grandis* bioactive compounds toward EGFR-1

	Ligand	Name	Category	Type	Binding energy (kcal/mol)
EGFR-1	Epigallocathecin 3-O-cinnamate	Arg841, Asn842	Hydrogen Bond	Conventional Hydrogen Bond	-9.1
		Leu718	Hydrophobic	Pi-Sigma	
		Val726, Ala743, Lys745	Hydrophobic	Alkyl	
		Lys745, Leu788, Val726	Hydrophobic	Pi-Alkyl	
	Epigallocatechin 3-O-p-coumarate	Lys745, Arg841	Hydrogen Bond	Conventional Hydrogen Bond	-8.8
		Cys797	Other	Pi-Sulfur	
		Val726	Hydrophobic	Alkyl	
		Val726, Leu718, Ala743, Lys745	Hydrophobic	Pi-Alkyl	
	Tectograndinol	Lys745, Thr854, Ala743	Hydrogen Bond	Conventional Hydrogen Bond	-7.3
		Val726, Ala743, Lys745, Leu844, Leu718, Cys797	Hydrophobic	Alkyl	
	Curcumin	Met793, Leu788	Hydrogen Bond	Conventional Hydrogen Bond	-8.4
		Gln791	Hydrogen Bond	Carbon Hydrogen Bond	
		Leu718	Hydrophobic	Pi-Sigma	
		Leu844, Val726	Hydrophobic	Pi-Alkyl	
	Nitrofurazone	Thr854, Asp855, Phe856	Hydrogen Bond	Conventional Hydrogen Bond	-6.2
		Phe856	Hydrogen Bond	Pi-Donor Hydrogen Bond	
		Val726, Ala743, Lys745	Hydrophobic	Pi-Alkyl	

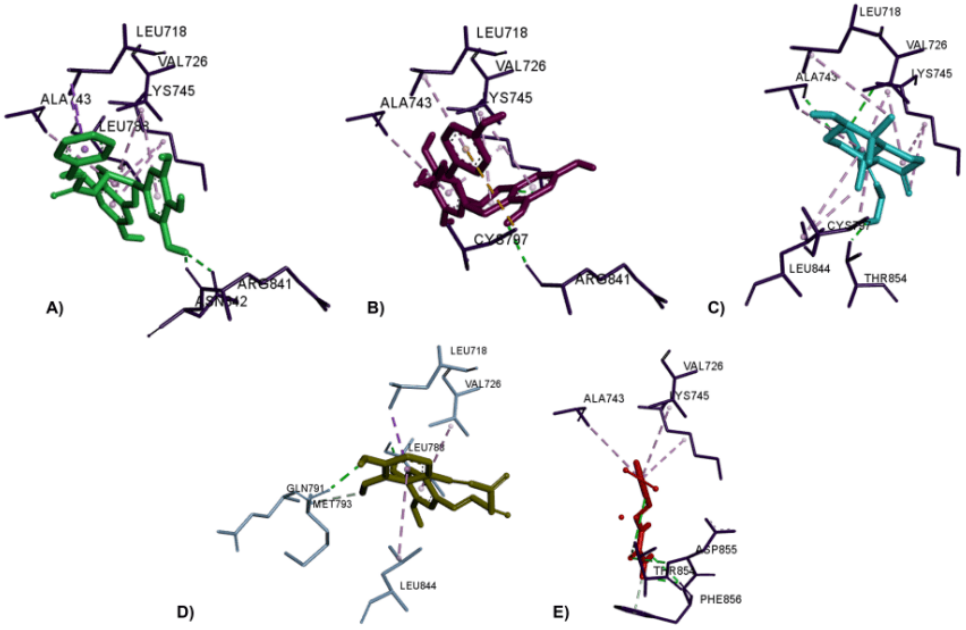

Figure 5 Interaction of EGFR-1 with ligands

Notes: A = epigallocathecin 3-O-cinnamate; B = epigallocatechin 3-O-p-coumarate; C = tectograndinol; D = curcumin; E = nitrofurazone. All bioactive compounds posed similar binding sites toward EGFR-1.

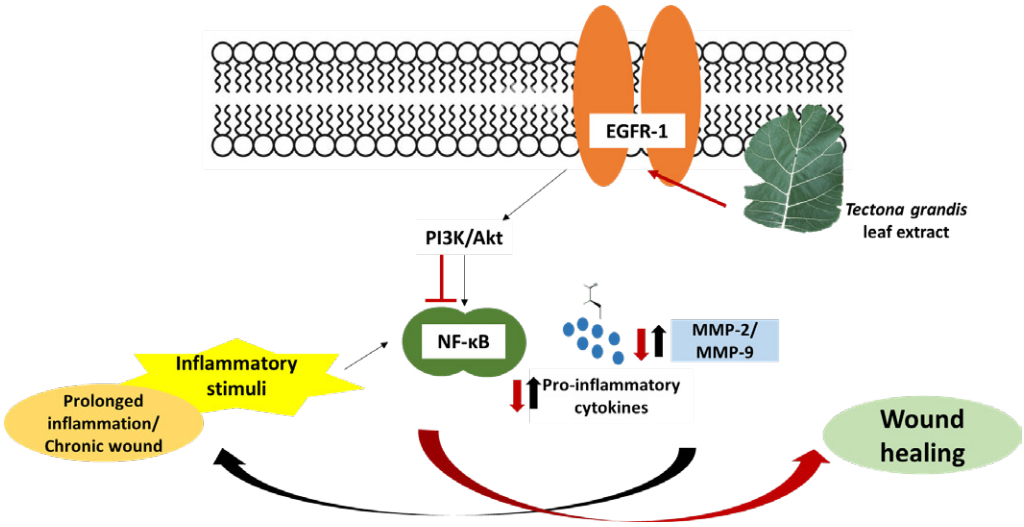

Figure 6 Prediction of the molecular mechanism of *T. grandis* bioactive compounds in promoting wound healing via EGFR-1 and NF-κB signaling, leading to regulated MMP-2/MMP-9 expression

During wound-healing process, NF-κB naturally activated as innate immune response in all cells as a response to infection or injury. It is essential for migration of phagocytic and inflammatory cells to the tissue. The binding of EGF and EGFR initiates the cell proliferation and migration by PI3K factors activation, JAK/STAT, PLC and PKC, also Ras/Raf signaling via EGF/EGFR pathway. The mechanism initiates upregulation of NF-κB signalling which stimulates prolonged inflammation (Bodnar 2013; Tomas *et al.* 2014; Yang *et al.* 2022).

The upregulated NF-κB may lead to impaired wound-healing process due to the role of NF-κB as precursor of inflammation. In addition, NF-κB activation also facilitate in upregulated MMP-9 expression, leading to prolonged inflammation and development of chronic wound (Ambrozova *et al.* 2017; Yadav *et al.* 2024). Moreover, MMP-2 and MMP-9 are jointly involved in the hemostasis process of wound-healing mechanism (Kandhwal *et al.* 2022). Expression of MMP-2 during inflammation triggers the MMP-9 in promoting cell migration and re-epithelialization (Caley *et al.*

2015; Lee & Kim 2022). However, upregulated MMP-2 and MMP-9 is associated with chronic wounds. In the vascular cells, overexpressed MMP-2 and MMP-9 promote ECM degradation (Ayuk *et al.* 2016; Hingorani *et al.* 2019). Thus, maintaining the expression of MMP-2 and MMP-9 is necessary to accelerate wound-healing process (Chiang *et al.* 2023).

The bioactive compounds from *T. grandis* leaves form a strong binding with the receptor, as suggested by the presence of hydrogen bonds. These bonds play a pivotal role in stabilizing the receptor-ligand complex and increasing the ligand's efficiency in performing its action (Krisnamurti *et al.* 2021). Wound care using *T. grandis* leaves extract could balance each wound healing phase, leading to acceleration of the healing process. The mechanism predicted from this study was that the bioactive compounds of *T. grandis* leaf act as MMP-2 inhibitor and MMP-9 inhibitor. It prevents the upregulation of MMP-2 and MMP-9 expression during inflammation phase in the wound-healing process, leading to the failure of chronic wound development. The controlled MMP-2 and MMP-9 expressions initiate the migration of fibroblast to the wound site via ECM remodeling (Hingorani *et al.* 2019). Moreover, bioactive compounds from *T. grandis* leaf have the potential to stimulate EGFR-1, as shown by molecular docking studies. This protein is identified for its pivotal role in wound-healing process by regulating cell proliferation, survival, and differentiation (Chakraborty *et al.* 2014; Sun *et al.* 2021). A previous study showed that mice treated with EGFR-1 exhibited a reduced incision wound width compared to the untreated group. Additionally, abundant hair growth was observed in the EGFR-treated mice without incision wounds, showing the importance of EGFR stimulation during the wound healing process (Repertinger *et al.* 2004). The binding of ligands to EGFR-1 may stimulate cell proliferation and migration via the PI3K, JAK/STAT, PLC, and PKC pathways, all of which aid the wound healing process (Tomas *et al.* 2014; Yang *et al.* 2022).

Our study suggested that the bioactive compounds of *T. grandis* leaf downregulate NF-κB expression, leading to anti-inflammatory activity. The mechanism also contributes in lowering expression of MMP-2 and MMP-9. The regulated expression of MMP-2 and MMP-9 would allow cell migration during the wound-healing process (Lee & Kim 2022). Treatment and therapy of wounds with *T. grandis* leaf could be an alternative treatment with high safety and effectiveness. However, the biological activity of *T. grandis* bioactive compounds in wound treatment should be further studied.

CONCLUSION

Epigallocatechin 3-O-cinnamate, epigallocatechin 3-O-p-coumarate, and tectograndinol were identified as the most abundant bioactive compounds from a total of 82 bioactive compounds contained in *T. grandis* methanolic extract. The bioactive compounds was predicted to have biological activities related to wound-healing process as antioxidant, free radical scavenger, anti-inflammatory, and MMP-9 expression inhibitor. In silico analysis of drug-likeness and ADME/T showed that *T. grandis* bioactive compounds possessed favorable properties and were predicted to be highly safe. This study suggested that bioactive compounds hold potential for developing new medication with reduced side effects. Further study by molecular docking demonstrated the wound-healing process via NF-κB, MMP-2, MMP-9, and EGFR-1 binding, which showed similar results as that of commonly prescribed wound-healing pharmaceuticals. This study predicted the molecular mechanism of wound-healing process using the bioactive compounds of *T. grandis* by performing inhibition of NF-κB, MMP-2, MMP-9 and stimulating EGFR-1. Further in vivo or in vitro studies are required to evaluate the activity of *T. grandis* bioactive compounds in wound treatment.

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