Biointerface Research in Applied Chemistry
Open-Access Journal (ISSN: 2069-5837)

Article
Volume 15, Issue 6, 2025, 88
https://doi.org/10.33263/BRIAC156.088

Wound Healing Potentials of Terpenoids from Malaysian Stingless Bee Propolis: Integrated Network Pharmacology and Molecular Docking Approaches

Md Shamim Rahman [1], Miah Roney [2], Firdaus Basrawi [3], Nurul Aini Mohd Azman [1,*]

[1] Faculty of Chemical and Process Engineering Technology, Universiti Malaysia Pahang Al-Sultan Abdullah, Lebuhraya Persiaran Tun Khalil Yaakob, Gambang 26300, Kuantan, Pahang, Malaysia
[2] Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang Al-Sultan Abdullah, Lebuhraya Persiaran Tun Khalil Yaakob, Gambang 26300, Kuantan, Pahang, Malaysia
[3] Faculty of Mechanical and Automotive Engineering Technology, Universiti Malaysia Pahang Al-Sultan Abdullah, Lebuhraya Persiaran Tun Khalil Yaakob, Gambang 26300, Kuantan, Pahang, Malaysia
* Correspondence: ainiazman@umpsa.edu.my;

Received: 24.02.2025; Accepted: 10.05.2025; Published: 14.11.2025

Abstract: Wounds offer major health concerns and cause a great deal of economic, financial, and social hardship for hospitals, patients, families, and caretakers worldwide. Wounds are said to result from natural, chemical, or thermal stressors that weaken the skin's structure. Therefore, developing novel medications for wound healing is crucial to address the gaps in managing various types of wounds effectively. This study employed molecular docking and network pharmacology to investigate the effects of these compounds on biological systems and assess the efficacy of stingless bee propolis terpenoids in wound care. Using STRING, Cytoscape 3.10.2, and DAVID software, a network pharmacology analysis was conducted, and a network of interaction targets related to wound healing was produced. GO, KEGG, and Reactome analyses were employed to identify the specific biological processes, cellular compartments, and molecular functions associated with wounds. Additionally, brief and preliminary molecular docking research was conducted to examine the interactions between the terpenoids of propolis from stingless bees and the target protein, which was selected based on its rank value. Based on network pharmacology analysis, 48 hub genes were identified as potential therapeutic targets and are implicated in the R-HSA-162582 Signal Transduction signaling pathway related to wound healing. Furthermore, α-Eudesmol showed the highest binding score for ESR1 (-8.1 kcal/mol), according to docking studies. This study suggests that α-Eudesmol may improve wound healing. These findings offer the chance to demonstrate the effectiveness of α-Eudesmol as a multi-target medication in wound patients through both in vitro and in vivo investigations.

Keywords: wound healing; ESR1; terpenoid; network pharmacology; docking.

1. Introduction

Wounds are a significant global medical issue that threatens the health systems [1]. The physiological process of wound healing is essential, dynamic, and intricate for maintaining skin integrity and repairing tissues. Angiogenesis, tissue growth, inflammation, and regeneration are all well-integrated steps that are required in wound healing [2]. It is proven that chronic inflammation retards the wound-healing process by interfering with the body's natural healing

mechanisms. A prolonged inflammatory phase can cause the wound to become chronic, delaying the regeneration and remodeling stages that are essential for healing. Since 1997, the FDA has approved Becaplermin, a gel that comprises platelet-derived growth factor (PDGF), for the treatment of diabetic foot ulcers [3,4]. Currently, no FDA-approved wound healing agent exists for the treatment of general wounds, underscoring the urgent need for effective and innovative therapeutic options. Every year, millions of people experience chronic wounds, which can lead to infections and tissue damage [5]. The prospect for novel medication is shown by the recent authorization of Vyjuvek, a gene therapy for dystrophic epidermolysis bullosa [6]. Notwithstanding these developments, it is high time to discover new drugs to heal wounds and meet the unmet demand for efficient treatment management of various types of wounds.

Herbal therapies are promising as a source of new treatment options needed for human well-being. These herbal drugs are the result of the creation of semi-synthetic and/or synthetic analogs, which are required for the therapy of specific diseases; they provide chemical scaffolds [7]. Over 500 species of stingless bees (Hymenoptera: Apidae) are included in the tribe Meliponini, which is found worldwide in tropical dry and wet forests, as well as in certain subtropical regions [8]. Stingless bees are classified into two genera: Melipona and Trigona. Due to their biological activity and significant contribution to the forest environment, these have been the subject of phylogeny, sociality, and colony evolutionary studies [9]. Other remarkable features of stingless bees are propolis, honey, and wax. For a long time, stingless bee honey has been consumed and utilized as a medicinal product due to its significant biologically active ingredients, including carotenoids, polyphenols, proteins, minerals, free amino acids, and vitamins [8]. Moreover, it has been proven that propolis has antiseptic properties [10]. Propolis has been demonstrated in several studies to possess antibacterial [11], antioxidant [12], anti-inflammatory [13], antifungal [14], and wound-healing [15] properties. The chemical constitution of stingless bee propolis, honey, and wax varies according to the bee species, storage conditions, environmental factors, and botanical origin. Propolis' biological activities have been ascribed to its diterpenic acid, flavonoid, phenolic, terpenoid, and aromatic acid contents [16].

Terpenoids are known for their diverse pharmacological properties, including antioxidant, antibacterial, and anti-inflammatory effects [17,18]. Terpenoids have the potential to improve wound healing by encouraging angiogenesis and re-epithelialization. For example, certain terpene saponins promote angiogenesis and the production of vascular endothelial growth factor (VEGF), two essential processes for tissue healing [19]. They are also useful ingredients in creating therapies that promote the coagulation, inflammation, proliferation, and remodeling stages of the wound healing cascade, due to their capacity to lower inflammation and fight infections [20]. Drug research efforts continue to be made to optimize delivery systems and determine appropriate dosages for therapeutic use, despite their potential advantages in wound care treatment. Given the wound-healing properties of stingless bee propolis [21,22] and terpenoids [23,24], we selected the terpenoids extracted from stingless bee propolis for this study.

Network pharmacology is an emerging approach in upstream drug discovery that enables systematic exploration of disease pathways and therapeutic targets [25]. By integrating existing data from various databases, a predictive model of drug action mechanisms can be constructed, linking compound targets with disease-related targets—such as those involved in wound healing. This approach helps identify pharmacological mechanisms within the broader context of biological network homeostasis [26]. Molecular docking complements network

pharmacology by allowing for the precise identification and optimization of potential drug candidates. Together, these computational techniques provide a faster and more cost-effective path for drug discovery, paving the way for novel treatment strategies. The primary aim of this study is to utilize network pharmacology and molecular docking to investigate the wound-healing potential of terpenoids derived from Malaysian stingless bee propolis. By identifying key bioactive compounds and their molecular targets, this research provides valuable scientific insights into natural therapies and supports the development of novel, effective, and safe wound-healing agents rooted in traditional medicine.

2. Materials and Methods

2.1. Source of targets.

In this study, five terpenoids—Germacrene D, Isolongifolol, Phorbol, α-Eudesmol, and Isoaromadendrene epoxide—were selected based on the findings of Syed Salleh et al. [29], who identified these compounds in stingless bee propolis using GC-MS analysis. The SMILES structures of terpenoids present in stingless bee propolis were retrieved and analyzed using the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The target for these drugs was predicted using the SwissTargetPrediction and SuperPred databases [27]. The duplicate targets were also eliminated to identify the unique targets.

2.2. Common target identification.

Then, using Venny 2.1.0 software, the Venn diagram of intersecting targets was created by intersecting the unique targets from the SwissTargetPrediction and SuperPred databases [28].

2.3. Construction of protein-protein interaction network.

The String network platform was used to import the intersecting targets for the terpenoids of propolis from stingless bees [27]. "*Homo sapiens*" was selected as the protein type. >0.9 was the chosen degree of confidence. The protein-protein interaction network (PPI) was created after the free targets were eliminated. The PPI was mapped using Cytoscape 3.10.2 software after the nodal relationship data were imported to determine the degree [29]. The degree was then used to predict the primary targets for terpenoids of stingless bee propolis in wound treatment.

2.4. Enrichment analysis.

The Kyoto Encyclopaedia of Genes and Genomes (KEGG) and Gene Ontology (GO) were used to clarify the roles of the target genes that were screened. The DAVID database was used for enrichment analysis, and $P < 0.05$ was used as the criterion for statistical significance [30]. Bubble charts were used to visually represent the results using the Microbiome platform (https://www.bioinformatics.com.cn/).

2.5 Molecular docking.

The target protein for docking was the crystal structure of the ESR1 protein, which has the PDB code 1X7R [31]. Five terpenoids from stingless bee propolis were docked with the ESR1 protein using the CB-Dock2 (Cavity-detection guided Blind Docking2) method [32]. In

this study, we utilized a blind docking tool (CB-Dock2), which requires a protein file in .pdb format and ligand files in .mol format. No additional preparation of the protein or ligands is necessary, as the software automatically selects the appropriate grid box based on the compound size [32]. These ligands were created using the ChemSketch program, stored in .mol format, and obtained from literature sources [33]. After rapidly identifying the binding site, determining the size and placement of the center, and customizing the docking zone's size based on the input molecules, the CB-Dock2 protein-ligand docking tool is used to dock using AutoDock Vina version 1.1.2 [34]. Before the docking process, a PBD file of the receptor and a .mol file containing the ligands were imported into the CB-Dock2 application. Some main cavities were automatically selected and utilized for further docking studies during this approach. The ideal binding position for the query ligand should correspond to its location, and the first conformation is considered to indicate the best binding site. In comparison to the reference compound (Cefuroxime), the best-docked posture was chosen as the lead compound after analyzing the binding modalities, interaction with active site residues, and docking score.

3. Results and Discussions

3.1. Source of targets.

402 targets for the terpenoids of stingless bee propolis were found using the SwissTargetPrediction database (Table S1), 226 of which are unique genes (Table S2). Additionally, 725 targets for the terpenoids of stingless bee propolis were found using the SuperPred database (Table S3), with 303 of them being unique genes (Table S4).

3.2. Common target identification.

The Venny program was used to enter 303 targets from the SuperPred database and 226 targets from the SwissTargetPrediction database. Following their intersection, 77 targets (Figure 1) for stingless bee propolis terpenoids for wound therapy were found (Table S5).

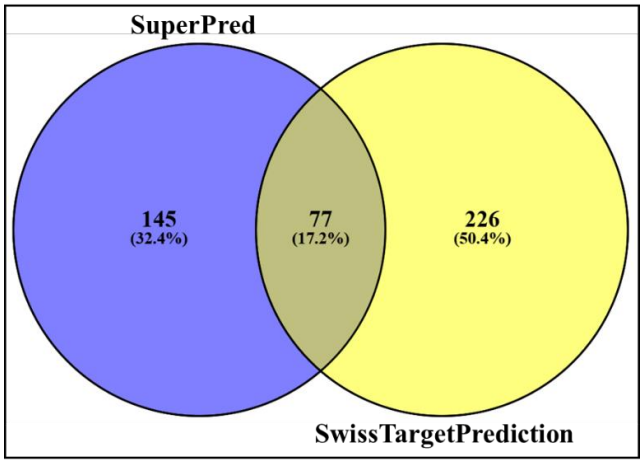


Figure 1. Network pharmacology of terpenoids in stingless bee propolis-related targets for treating wounds. Venn diagram of the common target at the intersection of terpenoids of stingless bee propolis from the SwissTargetPrediction and SuperPred databases.

3.3. Construction of protein-protein interaction network.

A comprehensive PPI network comprising 76 nodes and 264 edges was constructed by integrating the common target genes into the STRING platform (Figure 2 and Table S6). The network diagram was then loaded into Cytoscape 3.10.2 for image processing and network

diagram analysis to get fundamental network data. The graphic shows that the more connected a node is, the redder its color (Figure 3a). Key targets like ESR1 (Score: 22), OPRM1 (Score: 18), MAPK1 (Score: 17), HIF1A (Score: 16), GRM5 (Score: 16), MMP9 (Score: 14), PRKCA (Score: 14), MAOB (Score: 14), PIK3CA (Score: 14), and DRD1 (Score: 13) were among the top ten key genes in the PPI network that were filtered by rank value from the degree method using the CytoHubba plugin (Figure 3b).

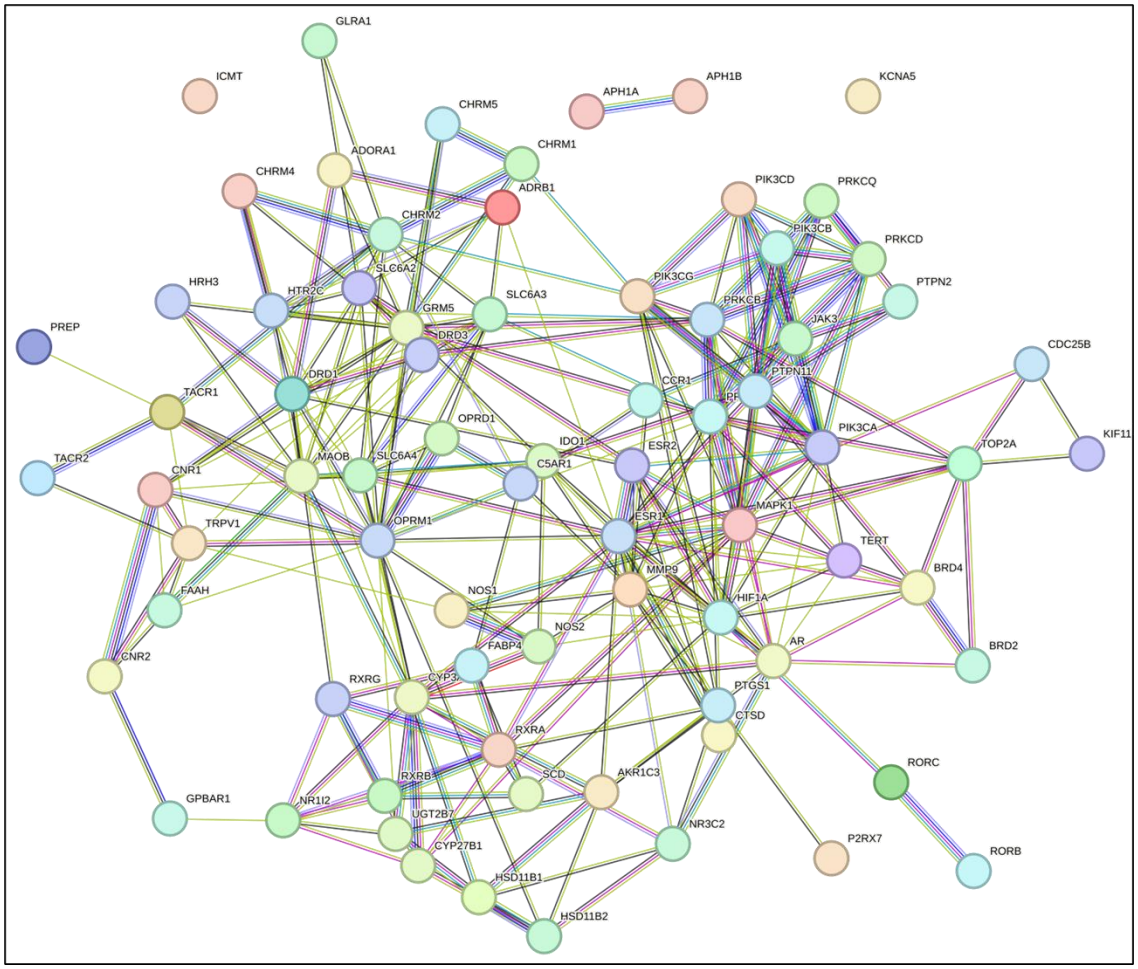


Figure 2. PPI plot of key targets of terpenoids of stingless bee propolis for wound healing using SRING Software.

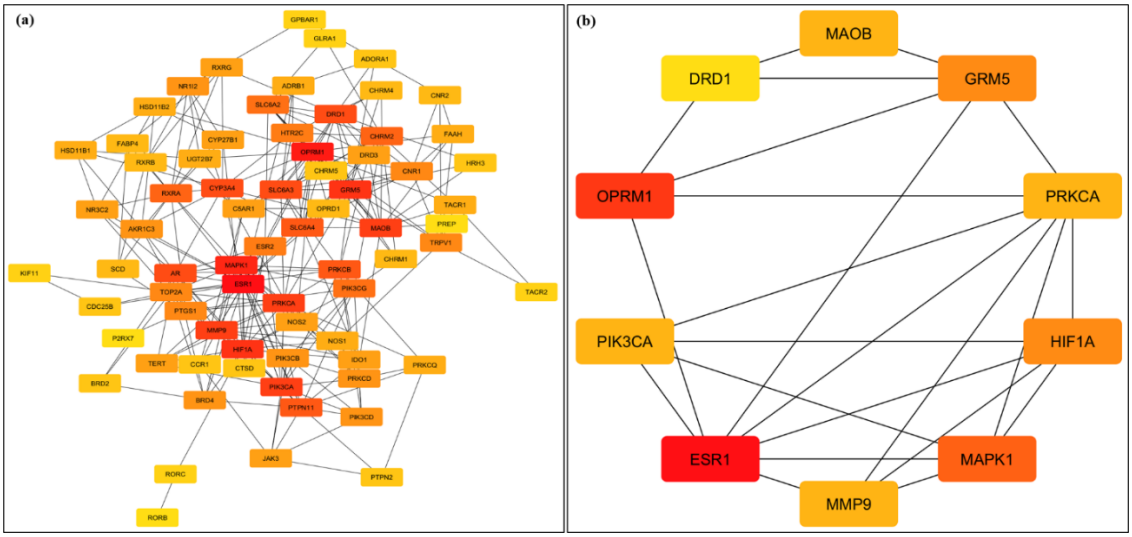


Figure 3. (a) PPI plot of key targets for terpenoids of stingless bee propolis for wound healing using Cytoscape 3.10.2 software; **(b)** Top 10 PPI plot using Cytoscape 3.10.2 software.

3.4. Enrichment analysis.

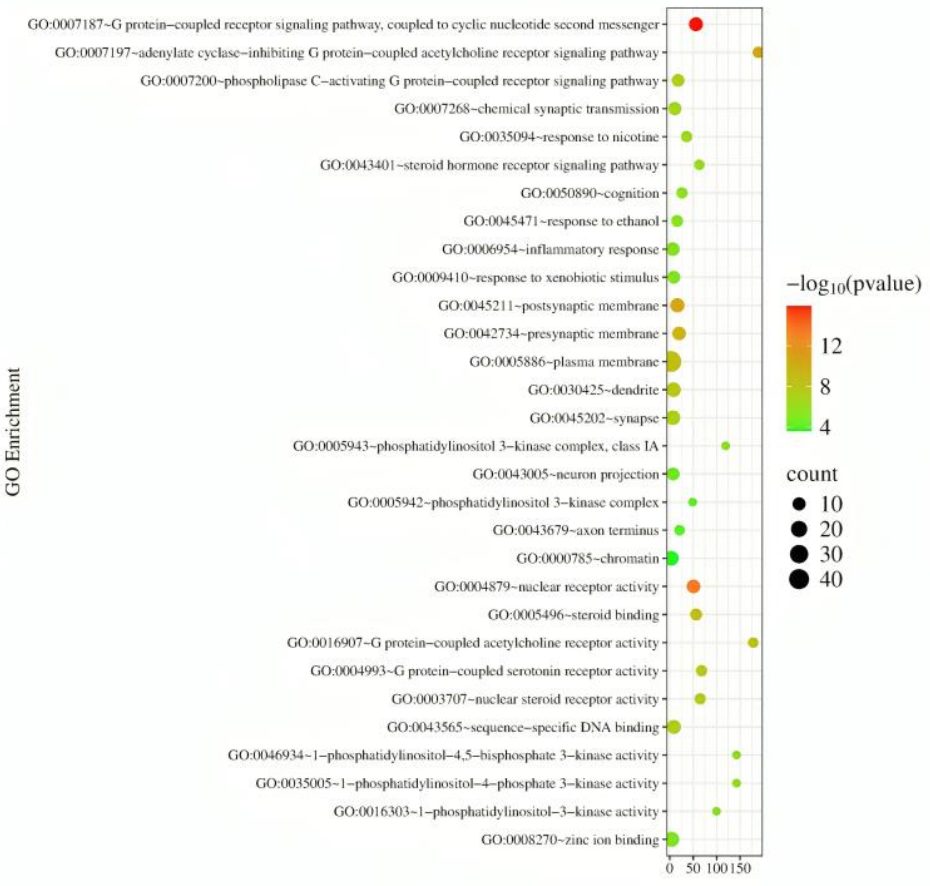
Using the DAVID databases, GO functional and KEGG pathway enrichment analyses were performed on the 77 frequent target genes. Overall, 223 biological activities (Table S7), 41 cellular components (Table S8), and 71 molecular functions (Table S9) were discovered. Adenylate cyclase-inhibiting G protein-coupled acetylcholine receptor signaling pathway, phospholipase C-activating G protein-coupled receptor signaling pathway, chemical synaptic transmission, response to nicotine, steroid hormone receptor signaling pathway, cognition, response to ethanol, inflammatory response, response to xenobiotic stimulus, etc., were the biological processes that were primarily involved in the G protein-coupled receptor signaling pathway, coupled to cyclic nucleotide second messenger. The main cellular components were the postsynaptic membrane, presynaptic membrane, plasma membrane, dendrite, synapse, phosphatidylinositol 3-kinase complex, class IA, neuron projection etc.. The molecular functions were mainly associated with nuclear receptor activity, steroid binding, G protein-coupled acetylcholine receptor activity, G protein-coupled serotonin receptor activity, nuclear steroid receptor activity, sequence-specific DNA binding, 1-phosphatidylinositol-4,5-bisphosphate 3-kinase activity, 1-phosphatidylinositol-4-phosphate 3-kinase activity, 1-phosphatidylinositol-3-kinase activity, zinc ion binding, estrogen response element binding, enzyme binding, diacylglycerol-dependent, calcium-independent serine/threonine kinase activity, calcium,diacylglycerol-dependent serine/threonine kinase activity, diacylglycerol-dependent serine/threonine kinase activity, etc (Figure 4a).

The KEGG pathway-enriched molecules were implicated in 120 pathways (Table S10), primarily associated with the following: Aldosterone-regulated sodium reabsorption, Cholinergic Synapse, Thyroid hormone signaling pathway, Calcium signaling pathway, Inflammatory mediator regulation of TRP channels, Oestrogen signaling pathway, Neuroactive ligand-receptor interaction, Chemical carcinogenesis-receptor activation, non-small cell lung cancer, etc. (Figure 4b). Additionally, the Reactome pathway-enriched were involved in 129 pathways (Table S11), primarily related to classes A/1 (Rhodopsin-like receptors), GPCR downstream signaling, GPCR signaling, signal transduction, nuclear receptor transcription pathway, amine ligand-binding receptors, G alpha (i) signaling events, SUMOylation of intracellular receptors, G alpha (q) signaling events, etc. (Figure 4c).

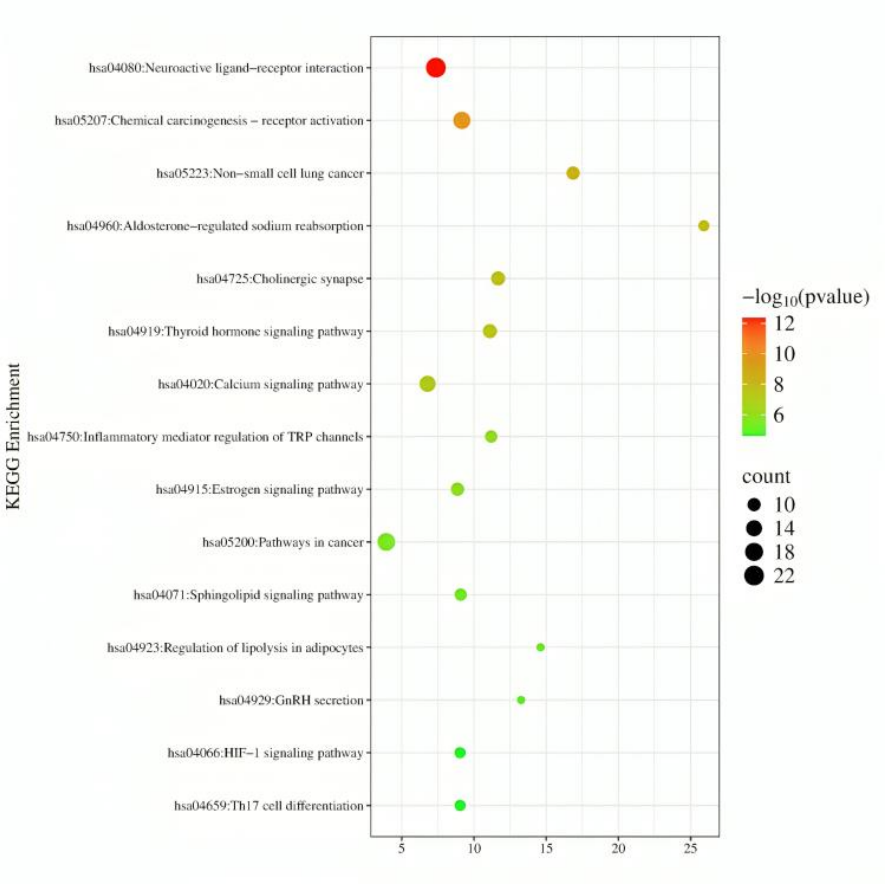
Topological characteristics were used to analyze the PPI network. The unique genes from the KEGG (top 10 pathways), Reactome (R-HSA-162582~Signal Transduction), and GO (top 10 pathways for BP, CC, and MF) were assessed for each topological parameter using DAVID software. After analyzing the degree value (rank value) in Cytoscape software, we identified top 10 hub (Figure 3b), which play a major role in the development of wound healing agents, including ESR1 (rank value: 22), OPRM1 (rank value: 18), MAPK1 (rank value: 17), HIF1A (rank value: 16), GRM5 (rank value: 16), MMP9 (rank value: 14), PRKCA (rank value: 14), MAOB (rank value: 14), PIK3CA (rank value: 14) and DRD1 (rank value: 13).

In addition, we discovered that 48 hub targets, such as CHRM2, OPRD1, CHRM1, CHRM4, C5AR1, CHRM5, HTR2C, PIK3CD, ADRB1, PIK3CB, HIF1A, PIK3CG, APH1A, RXRB, GRM5, APH1B, RXRA, TERT, CNR2, HRH3, CNR1, ADORA1, MAPK1, DRD1, JAK3, DRD3, CTSD, RXRG, CCR1, ICMT, PRKCB, PRKCD, AKR1C3, PTPN11, TACR1, MMP9, ESR1, ESR2, AR, PIK3CA, SCD, PRKCQ, and PTPN2 may be crucial in the R-HSA-162582~Signal Transduction signaling pathway with the terpenoids of stingless bee propolis (Figure 5). Based on the rank value, we selected the ESR1 (Estrogen Receptor 1) protein (rank

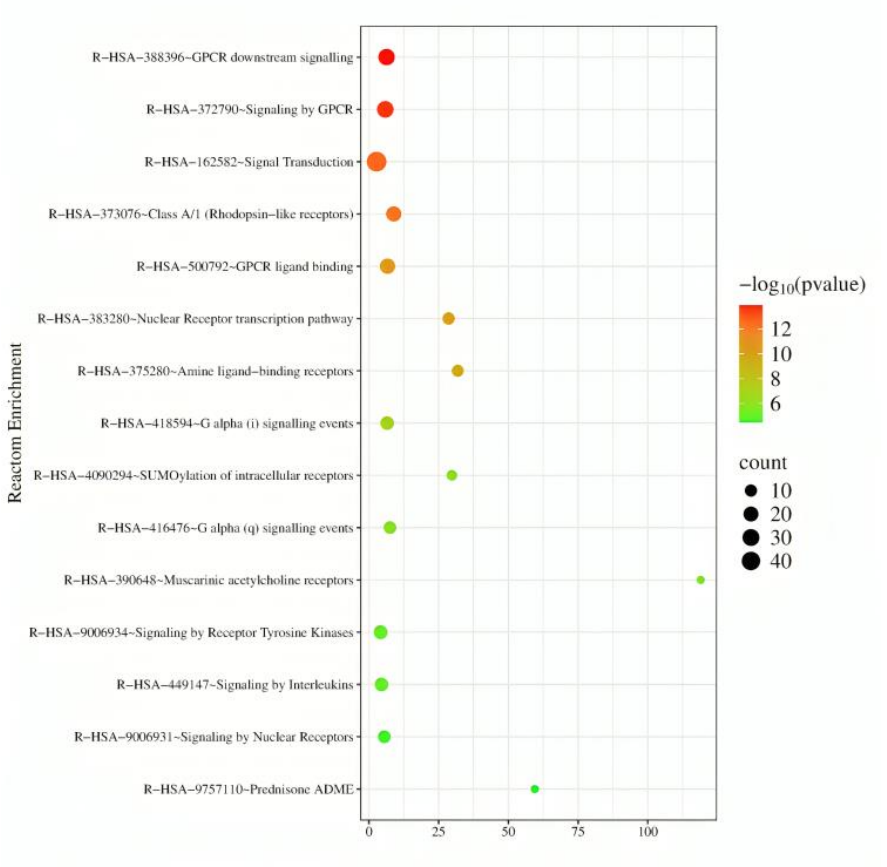
value: 22) for molecular docking to find an inhibitor against wounds from the terpenoids of stingless bee propolis after analyzing the rank values of these 48 hub targets (Figure 3b). The ESR1 gene plays a mechanistic role in the four phases of human wound repair: hemostasis, inflammation, proliferation, and remodeling. ESR1 influences the initial phase of wound healing by modulating the expression of genes involved in clot formation and vascular repair. Estrogen signaling pathways, mediated by ESR1, enhance platelet aggregation and fibrin clot stability, ensuring effective hemostasis [35]. During the inflammatory phase, ESR1 regulates the release of pro-inflammatory cytokines, such as IL-1β and TNF-α, which are critical for recruiting immune cells, including neutrophils and macrophages, to the wound site [35,36]. ESR1's modulation of estrogen signaling also affects oxidative stress and promotes anti-inflammatory responses, thereby reducing the prolonged inflammation commonly seen in chronic wounds. During the proliferation phase, ESR1 stimulates fibroblast activity and angiogenesis through the PI3K-Akt and MAPK signaling pathways. These pathways stimulate the deposition of extracellular matrix (ECM) and the production of vascular endothelial growth factor (VEGF), which are essential for tissue regeneration. Knockdown studies demonstrate that ESR1 inhibition impairs fibroblast proliferation and ECM interaction, underscoring its crucial role in tissue repair. ESR1 contributes to the final remodeling phase by regulating collagen synthesis and matrix metalloproteinases (MMPs), such as MMP2. This ensures proper ECM restructuring and scar formation. Additionally, ESR1's interaction with other hub genes, such as JUN and CD44, supports long-term tissue integrity and reduces pathological scarring. The mechanistic role of ESR1 across all phases highlights its potential as a therapeutic target for enhancing wound healing outcomes, particularly in conditions such as diabetic wounds, where its expression is dysregulated [35].



(a)



(b)



(c)

Figure 4. (a) GO functional enrichment analysis for terpenoids of stingless bee propolis in wound; **(b)** KEGG pathway enrichment analysis for terpenoids of stingless bee propolis in wound; **(c)** Reactome enrichment analysis for terpenoids of stingless bee propolis in wound.

The estrogen receptor (ER) is encoded by the ESR1 protein, which is important for wound healing and other physiological functions. The estrogen receptor itself is essential for regulating genes involved in wound repair, even though ESR1 mutations are mostly linked to breast cancer and resistance to endocrine therapy. Important elements of successful wound healing include matrix formation, inflammation, epidermal function, and protease inhibition, all of which are influenced by estrogen [37,38]. ESR1 has been recognized as a crucial gene in diabetic wounds (DWs) that may offer therapeutic options to enhance DW healing [38]. However, compared to its role in cancer therapy, the direct involvement of ESR1 mutations in identifying drugs for wound healing has received less attention. Rather, by affecting ER activity and associated pathways crucial for tissue regeneration, targeting coactivators such as SRC-3 may provide an indirect strategy [39]. All things considered, comprehending the intricate relationship between estrogen receptors and their coactivators may open up new possibilities for creating medications that promote the healing of wounds.

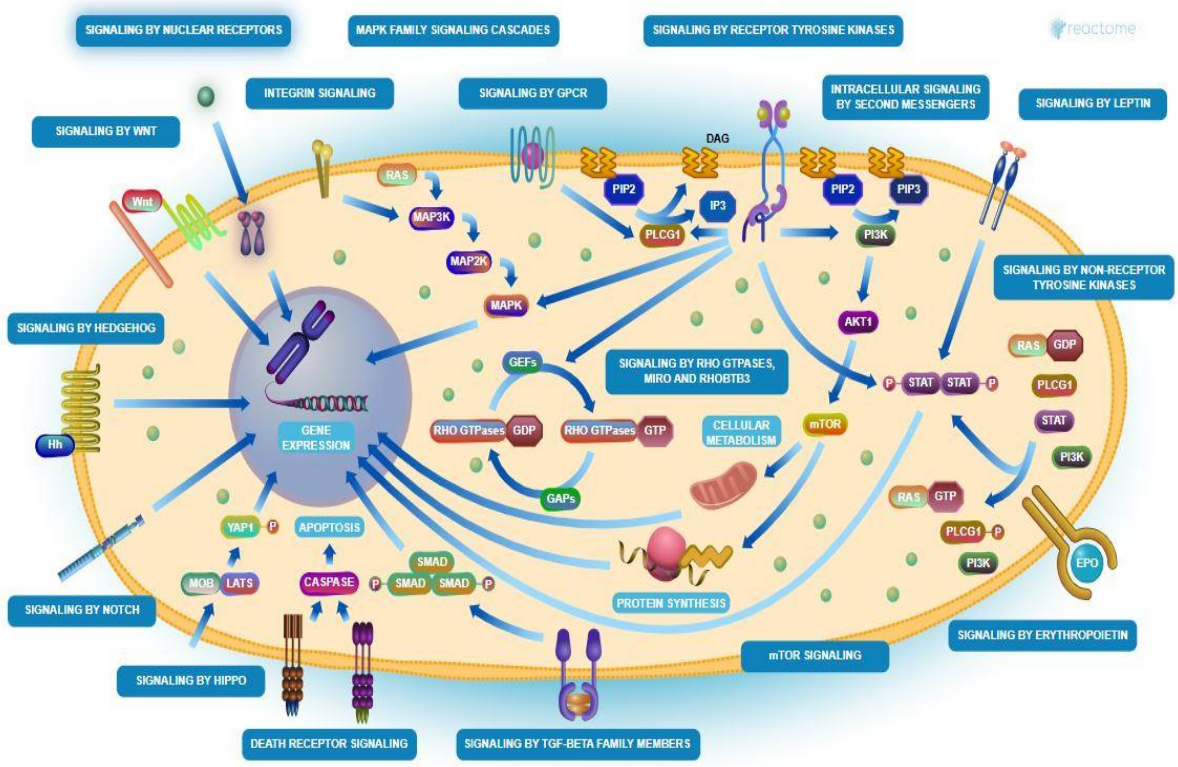


Figure 5. R-HSA-162582~Signal Transduction signaling pathway analysis for terpenoids of stingless bee propolis in wound.

Because they regulate several cellular functions, including inflammation, proliferation, and tissue remodeling, signal transduction pathways are essential for wound healing. The phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) pathway is a crucial signaling system that promotes angiogenesis and cell proliferation while reducing inflammation. Additionally, the Wnt/β-catenin pathway is important because it stimulates angiogenesis, migration, and cellular proliferation [40]. Furthermore, during wound healing, gasotransmitters such as hydrogen sulfide (H2S), carbon monoxide (CO), and nitric oxide (NO) influence epithelialization and migration, and exhibit anti-oxidative effects [41]. Developing tailored treatments for wound healing can be aided by an understanding of these signaling pathways. Modifying these pathways, for example, may lead to the development of medications that enhance wound closure by creating an environment conducive to tissue restoration. Additionally, new techniques to treat chronic wounds associated with diseases such as diabetes

mellitus may be provided by therapeutic strategies that employ gasotransmitters or other signaling molecules [42].

3.5. Molecular docking.

Because it can predict how well small molecules will bind to proteins involved in healing processes, molecular docking is crucial in the drug development process for wound healing. It aids in the discovery of potential therapeutic compounds and the optimization of their structures, thereby improving wound healing effectiveness by targeting key receptors and pathways [2, 43]. This strategy accelerates the creation of efficient wound care solutions.

Using the CB-Dock2 tool, the five terpenoids identified in stingless bee propolis were molecularly docked against the ESR1 protein, a key target involved in wound healing. CB-Dock2 utilizes a blind docking approach powered by AutoDock Vina, which automatically detects potential binding sites, calculates their size and center, and adjusts the docking box based on the input ligands. Due to this automated and precise configuration, no additional validation of the docking protocol is necessary. Consequently, CB-Dock can accelerate the docking procedure and improve accuracy by utilizing AutoDock Vina to predict the binding poses of query ligands and CurPocket, a curvature-based cavity identification technique, to predict the binding sites of target proteins. The compounds with the lowest docking energy value and the greatest number of interactions with active site residues were ranked higher than the control compound. As a result, the lead compound was determined to be the one that interacted and bound to the amino acid residues the best when compared to the control molecule. Nonetheless, the low docking energy value suggests that the connections were satisfactory [32].

All tested compounds successfully docked to the active site of the ESR1 protein, with binding energies ranging from –6.2 to –8.8 kcal/mol—values more favorable than that of the control compound (–6.8 kcal/mol), as presented in Table 1 and Table S12. Among them, α-Eudesmol exhibited the strongest binding affinity, forming seven pi-alkyl interactions with Ala350, Leu384, Leu387, Met388, Leu391, Phe404, and Leu428, in addition to one hydrogen bond with His525 (Figure 6a). In comparison, the control molecule displayed a pi-alkyl interaction with Leu349 and a pi–pi T-shaped interaction with Leu391 (Figure 6b). In this study, we used cefuroxime as a control or reference drug. Cefuroxime has shown strong potential in promoting wound healing, primarily due to its sustained antibacterial activity within wound exudates. In patients receiving vacuum-assisted therapy for fractures, systemic cefuroxime administration maintained drug concentrations above the minimum inhibitory concentration (MIC) for common pathogens such as *Staphylococcus aureus* and *Staphylococcus epidermidis*, resulting in high antibacterial efficacy—94.6% and 100%, respectively [44]. Moreover, the prophylactic use of cefuroxime has been associated with a notable reduction in surgical site infections, significantly lowering the incidence of wound sepsis compared to untreated controls [45]. Its rapid penetration into tissues ensures robust antimicrobial protection during surgical procedures, thereby supporting improved wound-healing outcomes [46].

Terpenoids like carvacrol and eugenol can successfully fight bacterial infections that often make wound healing more difficult by encouraging cell rupture and preventing protein synthesis [17].

Table 1. Molecular docking results analysis for terpenoids of stingless bee propolis with the ESR1 protein (PDB ID: 1X7R).

Compound name	Volume	Vina score	Interctions
Control (Cefuroxime)	391	-6.8	Phe404 (pi-pi T-shaped), Leu349, Leu391 (pi-alkyl)
Germacrene D	391	-7.9	Leu346, Ala350, Met388, Phe404 (alkyl/pi-alkyl)
α-Eudesmol	391	-8.1	His525 (H), Ala350, Leu384, Leu387, Met388, Leu391, Phe404, Leu428 (alkyl/pi-alkyl)
Isoaromadendrene epoxide	391	-8.8	Leu346, Ala350, Met388, Phe404, Leu525 (alkyl/pi-alkyl)

Furthermore, by promoting keratinocyte migration and proliferation—two essential processes in re-establishing tissue integrity following injury—terpenoids may accelerate the re-epithelialization process [19, 47]. In our investigation, the lead drug (α-Eudesmol) had a lower binding energy than the control molecule when it came to the ESR1 protein's active site.

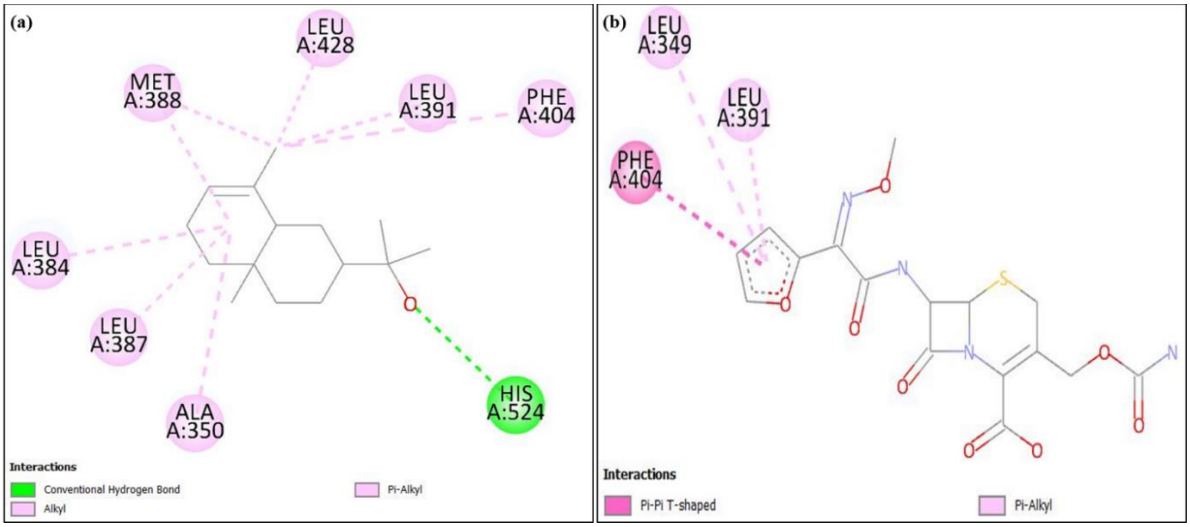


Figure 6. The interaction analysis of **(a)** lead compound (α-Eudesmol); **(b)** control compound (Cefuroxime) with the ESR1 protein (PDB ID: 1X7R).

4. Conclusions

This study employed bio-computational methods, including network pharmacology and molecular docking, to investigate interactions with key biological targets and demonstrate the therapeutic potential of stingless bee propolis terpenoids in wound treatment. The hypothesized biochemical processes and metabolic pathways affected by the terpenoids in stingless bee propolis were supported by a PPI network with 76 potential targets, as well as GO, KEGG, and Reactome pathway enrichment analysis. Most importantly, our research revealed that α-Eudesmol interacts strongly with the ESR1 protein, a crucial regulator in the pathophysiology of wounds and other diseases linked to aging. Because α-Eudesmol binds to the ESR1 protein so well, it could potentially affect wound healing. The practical implications of these findings are that α-Eudesmol may enhance wound management, thereby reducing adverse effects and complementing conventional wound care.

Author Contributions

Conceptualization, N.A.M.A.; methodology, M.S.R. and M.R..; validation, N.A.M.A. and F.B.; formal analysis, M.S.R.; investigation, N.A.M.A.; resources, M.R.; data curation, M.S.R.;

writing—original draft preparation, M.S.R. and M.R.; writing—review and editing, N.A.M.A and F.B.; visualization, M.S.R.; supervision, N.A.M.A.; project administration, N.A.M.A.; funding acquisition, N.A.M.A. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

The datasets analyzed during the current study are available as supplementary material accompanying this manuscript.

Funding

The study received financial support from Universiti Malaysia Pahang Al-Sultan Abdullah through internal university grants (Number: RDU233020 and PGRS 220316).

Acknowledgments

The authors would like to thank Universiti Malaysia Pahang, Al-Sultan Abdullah, Lebuhraya Persiaran Tun Khalil Yaakob, Gambang 26300, Kuantan, Pahang, Malaysia.

Conflicts of Interest

The authors declared that they have no conflict of interest.

References

1. Dong, R.; Guo, B. Smart wound dressings for wound healing. *Nano Today* **2021**, *41*, 101290, https://doi.org/10.1016/j.nantod.2021.101290.
2. Al Mousa, A.A.; Abouelela, M.E.; Mansour, A.; Nasr, M.; Ali, Y.H.; Al Ghamidi, N.S.; Abo-Dahab, Y.; Mohamed, H.; Abo-Dahab, N.F.; Hassane, A.M.A. Wound Healing, Metabolite Profiling, and In Silico Studies of *Aspergillus terreus*. *Curr. Issues Mol. Biol.* **2024**, *46*, 11681-11699, https://doi.org/10.3390/cimb46100694.
3. Patenall, B.L.; Carter, K.A.; Ramsey, M.R. Kick-Starting Wound Healing: A Review of Pro-Healing Drugs. *Int. J. Mol. Sci.* **2024**, *25*, 1304, https://doi.org/10.3390/ijms25021304.
4. Chen, M.; Chang, C.; Levian, B.; Woodley, D.T.; Li, W. Why Are There So Few FDA-Approved Therapeutics for Wound Healing?. *Int. J. Mol. Sci.* **2023**, *24*, 15109, https://doi.org/10.3390/ijms242015109.
5. Kathawala, M.H.; Ng, W.L.; Liu, D.; Naing, M.W.; Yeong, W.Y.; Spiller, K.L.; Van Dyke, M.; Ng, K.W. Healing of Chronic Wounds: An Update of Recent Developments and Future Possibilities. *Tissue Eng. Part B Rev.* **2019**, *25*, 429-444, https://doi.org/10.1089/ten.teb.2019.0019.
6. Khan, A.; Riaz, R.; Ashraf, S.; Akilimali, A. Revolutionary breakthrough: FDA approves Vyjuvek, the first topical gene therapy for dystrophic epidermolysis bullosa. *Ann. Med. Surg.* **2023**, *85*, 6298-6301, https://doi.org/10.1097/ms9.0000000000001422.
7. Shady, N.H.; Mostafa, N.M.; Fayez, S.; Abdel-Rahman, I.M.; Maher, S.A.; Zayed, A.; Saber, E.A.; Khowdiary, M.M.; Elrehany, M.A.; Alzubaidi, M.A.; Altemani, F.H.; Shawky, A.M.; Abdelmohsen, U.R. Mechanistic Wound Healing and Antioxidant Potential of *Moringa oleifera* Seeds Extract Supported by

Metabolic Profiling, In Silico Network Design, Molecular Docking, and In Vivo Studies. *Antioxidants* **2022**, *11*, 1743, https://doi.org/10.3390/antiox11091743.
8. Abdullah, N.A.; Zullkiflee, N.; Zaini, S.N.Z.; Taha, H.; Hashim, F.; Usman, A. Phytochemicals, mineral contents, antioxidants, and antimicrobial activities of propolis produced by Brunei stingless bees *Geniotrigona thoracica*, *Heterotrigona itama*, and *Tetrigona binghami*. *Saudi J. Biol. Sci.* **2020**, *27*, 2902-2911, https://doi.org/10.1016/j.sjbs.2020.09.014.
9. Choudhari, M.K.; Punekar, S.A.; Ranade, R.V.; Paknikar, K.M. Antimicrobial activity of stingless bee (*Trigona* sp.) propolis used in the folk medicine of Western Maharashtra, India. *J. Ethnopharmacol.* **2012**, *141*, 363-367, https://doi.org/10.1016/j.jep.2012.02.047.
10. Çelemli, Ö.G. Chemical Properties of Propolis Collected by Stingless Bees. In Pot-Honey: A legacy of stingless bees, Vit, P., Pedro, S.R.M., Roubik, D., Eds.; Springer New York: New York, NY, **2013**; pp. 525-537, https://doi.org/10.1007/978-1-4614-4960-7_39.
11. bdullah, N.A.; Ja'afar, F.; Yasin, H.M.; Taha, H.; Petalcorin, M.I.R.; Mamit, M.H.; Kusrini, E.; Usman, A. Physicochemical analyses, antioxidant, antibacterial, and toxicity of propolis particles produced by stingless bee *Heterotrigona itama* found in Brunei Darussalam. *Heliyon* **2019**, *5*, e02476, https://doi.org/10.1016/j.heliyon.2019.e02476.
12. Sawaya, A.; Calado, J.C.P.; Santos, L.C.d.; Marcucci, M.C.; Akatsu, I.P.; Soares, A.E.E.; Abdelnur, P.V.; Cunha, I.B.d.S.; Eberlin, M.N. Composition and antioxidant activity of propolis from three species of Scaptotrigona stingless bees. *J. ApiProduct ApiMed. Sci.* **2009**, *1*, 37-42.
13. Campos, J.F.; Santos, U.P.d.; Rocha, P.d.S.d.; Damião, M.J.; Balestieri, J.B.P.; Cardoso, C.A.L.; Paredes-Gamero, E.J.; Estevinho, L.M.; de Picoli Souza, K.; Santos, E.L.d. Antimicrobial, Antioxidant, Anti-Inflammatory, and Cytotoxic Activities of Propolis from the Stingless Bee *Tetragonisca fiebrigi* (Jataí). *Evid.-Based Complement. Altern. Med.* **2015**, *2015*, 296186, https://doi.org/10.1155/2015/296186.
14. Shehu, A.; Ismail, S.; Rohin, M.A.K.; Harun, A.; Abd Aziz, A.; Haque, M. Antifungal properties of Malaysian Tualang honey and stingless bee propolis against *Candida albicans* and *Cryptococcus neoformans*. *J. Appl. Pharm. Sci.* **2016**, *6*, 044-050, https://dx.doi.org/10.7324/JAPS.2016.60206.
15. Huanbutta, K.; Sittikijyothin, W.; Sangnim, T. Development of topical natural based film forming system loaded propolis from stingless bees for wound healing application. *J. Pharm. Investig.* **2020**, *50*, 625-634, https://doi.org/10.1007/s40005-020-00493-w.
16. El-Guendouz, S.; Lyoussi, B.; Miguel, M.G. Insight on Propolis from Mediterranean Countries: Chemical Composition, Biological Activities and Application Fields. *Chem. Biodivers.* **2019**, *16*, e1900094, https://doi.org/10.1002/cbdv.201900094.
17. Masyita, A.; Mustika Sari, R.; Dwi Astuti, A.; Yasir, B.; Rahma Rumata, N.; Emran, T.B.; Nainu, F.; Simal-Gandara, J. Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. *Food Chem. X* **2022**, *13*, 100217, https://doi.org/10.1016/j.fochx.2022.100217.
18. Câmara, J.S.; Perestrelo, R.; Ferreira, R.; Berenguer, C.V.; Pereira, J.A.M.; Castilho, P.C. Plant-Derived Terpenoids: A Plethora of Bioactive Compounds with Several Health Functions and Industrial Applications—A Comprehensive Overview. *Molecules* **2024**, *29*, 3861, https://doi.org/10.3390/molecules29163861.
19. Shedoeva, A.; Leavesley, D.; Upton, Z.; Fan, C. Wound Healing and the Use of Medicinal Plants. *Evid.-Based Complement. Altern. Med.* **2019**, *2019*, 2684108, https://doi.org/10.1155/2019/2684108.
20. Criollo-Mendoza, M.S.; Contreras-Angulo, L.A.; Leyva-López, N.; Gutiérrez-Grijalva, E.P.; Jiménez-Ortega, L.A.; Heredia, J.B. Wound Healing Properties of Natural Products: Mechanisms of Action. *Molecules* **2023**, *28*, 598, https://doi.org/10.3390/molecules28020598.
21. Manginstar, C.O.; Tallei, T.E.; Salaki, C.L.; Niode, N.J.; Jaya, H.K. Dual anti-inflammatory and antimicrobial effects of stingless bee propolis on second-degree burns. Narra J **2025**, 5, e2359., https://doi.org/10.52225/narra.v5i1.2359.
22. Ferreira, S.R.L.; Teixeira, S.A.; Lima, G.O.; de Castro, J.N.R.S.; Teixeira, L.E.O.; Barros, C.A.R.; Pereira, D.S.; Hamoy, M.; Bahia, V.R.L.O.; Muto, N.A. Healing Activity of Propolis of Stingless Bee (*Scaptotrigona aff. postica*), Reared in Monoculture of Açaí (*Euterpe oleracea*), in Induced Wounds in Rats. *Molecules* **2024**, *29*, 4742, https://doi.org/10.3390/molecules29194742.
23. Marinelli, L.; Cacciatore, I.; Eusepi, P.; Dimmito, M.P.; Di Rienzo, A.; Reale, M.; Costantini, E.; Borrego-Sánchez, A.; García-Villén, F.; Viseras, C.; Morroni, G.; Fioriti, S.; Brescini, L.; Di Stefano, A. In Vitro

Wound-Healing Properties of Water-Soluble Terpenoids Loaded on Halloysite Clay. *Pharmaceutics* **2021**, *13*, 1117, https://doi.org/10.3390/pharmaceutics13081117.
24. Villegas, L.F.; Marçalo, A.; Martin, J.; Fernández, I.D.; Maldonado, H.; Vaisberg, A.J.; Hammond, G.B. (+)-*epi*-α-Bisbolol Is the Wound-Healing Principle of *Peperomia galioides*: Investigation of the in Vivo Wound-Healing Activity of Related Terpenoids. *J. Nat. Prod.* **2001**, *64*, 1357-1359, http://dx.doi.org/10.1021/np0102859.
25. Singh, A.K.; Kumar, P.; Mishra, S.K.; Tiwari, K.; Singh, A.K.; Pandey, A.K.; Shati, A.A.; Alfaifi, M.Y.; Elbehairi, S.I.; Sayyed, R.Z. A network pharmacology approach with experimental validation to discover protective mechanism of poly herbal extract on diabetes mellitus. *J. King Saud Univ. Sci.* **2024**, *36*, 103138, https://doi.org/10.1016/j.jksus.2024.103138.
26. Di, S.; Han, L.; An, X.; Kong, R.; Gao, Z.; Yang, Y.; Wang, X.; Zhang, P.; Ding, Q.; Wu, H.; Wang, H.; Zhao, L.; Tong, X. *In silico* network pharmacology and *in vivo* analysis of berberine-related mechanisms against type 2 diabetes mellitus and its complications. *J. Ethnopharmacol.* **2021**, *276*, 114180, https://doi.org/10.1016/j.jep.2021.114180.
27. Wang, S.; Chen, W.; Dong, C.; Wu, J.; Zheng, M.; Ma, Y.; Xue, Y. Exploring the mechanism of genistein in treating hepatocellular carcinoma through network pharmacology and molecular docking. *Oncologie* **2024**, *26*, 799-811, https://doi.org/10.1515/oncologie-2024-0304.
28. Li, J.; Wang, X.; Xue, L.; He, Q. Exploring the therapeutic mechanism of curcumin in prostate cancer using network pharmacology and molecular docking. *Heliyon* **2024**, *10*, e33103, https://doi.org/10.1016/j.heliyon.2024.e33103.
29. Zhang, X.; Cao, J.-F.; Liao, D.; Xia, Z.; Xiong, L.; Wu, M.; Wang, C.; Yang, X.; Qiu, Y.; Zhang, L.; Chen, N.; Zhang, M.; Xia, Q. Dihydroartemisinin regulates the apoptosis and growth of colorectal cancer by suppressing DPYSL2 and increasing TF and ACHE. *Food Biosci.* **2024**, *61*, 104668, https://doi.org/10.1016/j.fbio.2024.104668.
30. Zhou, Z.; Li, Y.; Hao, H.; Wang, Y.; Zhou, Z.; Wang, Z.; Chu, X. Screening Hub Genes as Prognostic Biomarkers of Hepatocellular Carcinoma by Bioinformatics Analysis. *Cell Transplant.* **2019**, *28*, 76S-86S, https://doi.org/10.1177/0963689719893950.
31. Manas, E.S.; Xu, Z.B.; Unwalla, R.J.; Somers, W.S. Understanding the Selectivity of Genistein for Human Estrogen Receptor-β Using X-Ray Crystallography and Computational Methods. *Structure* **2004**, *12*, 2197-2207, https://doi.org/10.1016/j.str.2004.09.015.
32. Roney, M.; Huq, A.K.M.M.; Aluwi, M.F.F.M.; Tajuddin, S.N. *In-silico* Design of Curcumin Analogs as Potential Inhibitors of Dengue Virus NS2B/NS3 Protease. *J. Comput. Biophys. Chem.* **2023**, *22*, 645-653, https://doi.org/10.1142/S2737416523500321.
33. Syed Salleh, S.N.A.; Mohd Hanapiah, N.A.; Ahmad, H.; Wan Johari, W.L.; Osman, N.H.; Mamat, M.R. Determination of Total Phenolics, Flavonoids, and Antioxidant Activity and GC-MS Analysis of Malaysian Stingless Bee Propolis Water Extracts. *Scientifica* **2021**, *2021*, 3789351, https://doi.org/10.1155/2021/3789351.
34. Liu, Y.; Grimm, M.; Dai, W.-T.; Hou, M.-C.; Xiao, Z.-X.; Cao, Y. CB-Dock: a web server for cavity detection-guided protein–ligand blind docking. *Acta Pharmacol. Sin.* **2020**, *41*, 138-144, https://doi.org/10.1038/s41401-019-0228-6.
35. Qi, S.; Han, Q.; Xing, D.; Qian, L.; Yu, X.; Ren, D.; Wang, H.; Chen, Q. Functional Analysis of Estrogen Receptor 1 in Diabetic Wound Healing: A Knockdown Cell-Based and Bioinformatic Study. *Med. Sci. Monit.* **2020**, *26*, e928788, https://doi.org/10.12659/msm.928788.
36. Eming, S.A.; Martin, P.; Tomic-Canic, M. Wound repair and regeneration: Mechanisms, signaling, and translation. *Sci. Transl. Med.* **2014**, *6*, 265sr6, https://doi.org/10.1126/scitranslmed.3009337.
37. Ko, R.F.; Davidson, O.Q.C.; Ahmed, M.A.; Clark, R.M.; Brandenburg, J.S.; Pankratz, V.S.; Sharma, G.; Hathaway, H.J.; Prossnitz, E.R.; Howdieshell, T.R. GPER deficiency impedes murine myocutaneous revascularization and wound healing. *Sci. Rep.* **2024**, *14*, 18400, https://doi.org/10.1038/s41598-024-68620-3.
38. Al Mamun, A.; Shao, C.; Geng, P.; Wang, S.; Xiao, J. Recent advances in the role of neuroregulation in skin wound healing. *Burns & Trauma* **2025**, *13*, tkae072. https://doi.org/10.1093/burnst/tkae072.
39. Reinert, T.; Saad, E.D.; Barrios, C.H.; Bines, J. Clinical Implications of ESR1 Mutations in Hormone Receptor-Positive Advanced Breast Cancer. *Front. Oncol.* **2017**, *7*, 26, https://doi.org/10.3389/fonc.2017.00026.

40. Bonnici, L.; Suleiman, S.; Schembri-Wismayer, P.; Cassar, A. Targeting Signalling Pathways in Chronic Wound Healing. *Int. J. Mol. Sci.* **2024**, *25*, 50, https://doi.org/10.3390/ijms25010050.
41. Krizanova, O.; Penesova, A.; Sokol, J.; Hokynkova, A.; Samadian, A.; Babula, P. Signaling pathways in cutaneous wound healing. *Front. Physiol.* **2022**, *13*, 1030851, https://doi.org/10.3389/fphys.2022.1030851.
42. Hao, M.-Y.; Li, H.-J.; Han, H.-S.; Chu, T.; Wang, Y.-W.; Si, W.-R.; Jiang, Q.-Y.; Wu, D.-D. Recent advances in the role of gasotransmitters in necroptosis. *Apoptosis* **2025**, *30*, 616-635, https://doi.org/10.1007/s10495-024-02057-x.
43. Zhang, T.; Yang, F.; Qin, X.; Yang, X.; Zhang, C.; Wan, Z.; Lin, H. Investigation of the In Vivo, In Vitro, and In Silico Wound Healing Potential of *Pinctada martensii* Purified Peptides. *Mar. Drugs* **2022**, *20*, 417, https://doi.org/10.3390/md20070417.
44. Bischoff, M.; Beck, A.; Frei, P.; Bischoff, G. Pharmacokinetics of Cefuroxime in Traumatic Wound Secretion and Antibacterial Activity Under Vacuum Therapy. *J. Chemother.* **2010**, *22*, 92-97, https://doi.org/10.1179/joc.2010.22.2.92.
45. Croton, R.S.; Sykes, D.; Treanor, J.; Wake, P.; Green, H.T.; Knowles, M.A.; Eilon, L.A. The evaluation of cefuroxime in the prevention of postoperative infection. *Postgrad. Med. J.* **1981**, *57*, 363-365, https://doi.org/10.1136/pgmj.57.668.363.
46. Jørgensen, A.R.; Hanberg, P.; Bue, M.; Hartig-Andreasen, C.; Jørgensen, N.P.; Stilling, M. Local cefuroxime tissue concentrations in the hand after single and repeated administration to 16 patients undergoing trapeziectomy: a randomized controlled trial. *Acta Orthop.* **2024**, *95*, 498–504, https://doi.org/10.2340/17453674.2024.41343.
47. Pastar, I.; Stojadinovic, O.; Yin, N.C.; Ramirez, H.; Nusbaum, A.G.; Sawaya, A.; Tomic-Canic, M. Epithelialization in wound healing: a comprehensive review. *Advances in wound care* **2014**, *3*, 445-464. https://doi.org/10.1089/wound.2013.0473.

Publisher's Note & Disclaimer

Supplementary materials

Click here