



# Multi-target mechanism of polyherbal extract to treat diabetic foot ulcer based on network pharmacology and molecular docking



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## ABSTRACT

**Introduction:** Diabetic foot ulcer (DFU) potentially leads to loss of function, infections, hospitalization, lower-extremity amputation, and even death. The potential therapeutic efficacy of a polyherbal candidate named TIP-Heal was identified for treating DFU. TIP-Heal, which stands for *Tinospora crispa*, *Isotoma longiflora*, and *Piper betle* L var *nigra*, consists of extracts from these three herbs in a ratio of 2:1:1. The Indonesian population commonly uses these herbs due to their wound-healing properties. It is our interest to analyse the mechanism of the polyherbal extract using network pharmacology and molecular docking.

**Methods:** This study uses network pharmacology and molecular docking methods to analyze the multi-target mechanism of active compounds in TIP-Heal extract for DFU treatment. The proteins targeted by the bioactive chemical present in TIP-Heal and DFU were identified within a particular dataset with the keyword "homo sapiens." The identified target proteins were assessed using gene ontology (GO) analysis, the Kyoto Encyclopaedia of Gene and Genomes (KEGG) pathways, protein-protein interactions (PPIs), and molecular docking.

**Results:** The critical proteins obtained were AKT serine/threonine kinase 1 (AKT1), caspase-3 (CASP3), epidermal growth factor receptor (EGFR), proto-oncogene tyrosine-protein kinase Src (SRC) and matrix metalloproteinase-9 (MMP-9). Several compounds, namely PubChem (Compound Identifier=CID: 5319898), 3-epiursolic acid, palmitic acid, and alpha-linolenic acid showed great potential as viable candidates to facilitate the healing process of DFU.

**Conclusion:** The findings of this study indicate that the TIP-Heal extract has the potential to be used as a natural herbal treatment for DFUs with the involvement of AKT1, CASP3, EGFR, and SRC proteins.

### Implication for health policy/practice/research/medical education:

This study can provide preliminary information on the active ingredients in the polyherbal "TIP-Heal" and its potential molecular mechanisms for treating diabetic foot ulcers.

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## Introduction

One of the primary sources of preventable morbidity in individuals with diabetes is diabetic foot ulcer (DFU). This condition potentially leads to loss of function, infections, hospitalization, lower-extremity amputation, and even death (1). In addition, the International Diabetes Federation (IDF) Diabetes Atlas reported that 10.5%

of adults aged 20-79 years had diabetes (2). The global prevalence of DFU is approximately 6.3%, with regional variations (3). Furthermore, morbidity after ulceration is high, with a recurrence rate of 65% within 3-5 years and a lifetime incidence of lower limb amputation of 20% (1), while 40.3% of patients who had recurrent/new ulcers had a higher neuropathy incidence (4).

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There are three forms of DFU: diabetic peripheral neuropathy (DPN), peripheral artery disease (PAD), and mixed neuro-ischemic, with a prevalence of 35%, 15%, and 50%, respectively (5). The DPN and PAD risk factors include age, diabetes duration, and high level of hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) (6). In this context, diabetes-related PAD-associated vascular problems lead to perfusion deficit, which decreases the blood flow to the infected tissue, resulting in considerably bad infected ulcer clearance (7,8). Furthermore, acute hyperglycemia impairs the innate immune responses that ordinarily guard against infection (7).

Wound healing is frequently mediated by growth factors and cytokines generated by cells stimulated through immune response (9). Long-term high glucose levels cause aberrant expression of several molecules, including vascular endothelial growth factor (VEGF), hypoxia-inducible factor 1 (HIF1), and anti-inflammatory factors (10). This leads to bad angiogenesis and prolonged inflammation (9). Although there are numerous diabetic wound therapies available, only a few can improve oxygen delivery at the microvascular level (8).

Several proteins are associated with the pathophysiology of wound healing; matrix metalloproteinases (MMPs) have been linked to extend inflammation and imbalances in the extracellular matrix (ECM) formation and breakdown (11). In comparison, the pro-inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor- $\alpha$  (TNF $\alpha$ ), and the chemokine stromal cell-derived factor affect wound healing phases (12).

There are several drugs for DFU therapy approved by the US Food and Drug Administration (FDA), including growth factor agonists (CT-102 = Curative Technologies, Becaplermin), synthetic drugs such as Hyaff, wound healing matrix (13), and topical antibiotics. Some growth factor therapies are expensive and have not been proven to accelerate DFU healing, while the use of antibiotics increases resistance (7).

This current study was focused on a polyherbal extract for wound healing in DFU, containing a combination of *Tinospora crispa*, *Isotoma longiflora*, and *Piper betle* L var *nigra* extracts, called TIP-heal, which stands for the name of the three herbals. The individual herbal extract has been empirically used for DFU healing with its specific activities. *T. crispa* is used for treating diabetes, hypertension, and wound dressing (14,15). The active ingredients include apigenin, diosmetin, luteolin4'-methylether7glucoside, and syringin (14). While, *I. longiflora* contains lobeline and lobetyolin, which are used for the treatment of atherosclerosis, hypertension (16), diabetic retinopathy, anti-cancer, analgesic, sore throat, anti-inflammatory, and wound healing (17). The active ingredients of *Piper betle* L var *nigra* consist of 2-octenoic and 2-hexenoic acid (18), while its bioactivities include anti-cancer, anti-allergic, anti-bacterial, antioxidant, anti-diabetic, and wound healing (19).

The combination of these 3 types of herbs aims to obtain a synergistic effect that can be obtained from the main effects of each of these herbs. Apart from conducting in vivo tests, the authors want to first examine the potential of this combination in wound healing through network pharmacology and molecular docking. It is hoped that valuable information will be obtained in the development of polyherbals as wound healing agents and ultimately be able to determine clinically appropriate therapy. Therefore, we conducted an analysis to determine the multi-target mechanism of TIP-Heal extract to treat DFU.

## Materials and Methods

### Identification of bioactive compounds in TIP-Heal extract and their respective targets

The bioactive compounds in TIP-Heal extract were identified using the keywords "*Tinospora crispa*", "*Hippobroma longiflora*", and "*Piper betle*" on the CMAUP (collective molecular activities of useful plants) (20) (<https://bidd.group/CMAUP/>) and PubMed (<https://pubmed.ncbi.nlm.nih.gov/>) databases. Each candidate's 3D structural image was available from the PubChem database (<http://pubchem.ncbi.nlm.nih.gov/>). Furthermore, the canonical SMILES (The simplified molecular-input line-entry system) were submitted to the Swiss Target Prediction database (<http://swisstargetprediction.ch/index.php>) to assess their potential interaction with a protein target. Interesting proteins were obtained by downloading those with a probability value exceeding zero, after applying a filter using the taxonomic phrase "homo sapiens".

### Prediction of DFU-related target for TIP-Heal active ingredients

The GeneCards (<https://www.genecards.org>), DisGeNET (<https://www.disgenet.org>), and Therapeutic target database (<https://db.idrblab.net/ttd/>) were applied to acquire all target genes linked with DFU. In the end, a Venn diagram (<http://www.interactivenn.net/>) (21) was created to illustrate the overlapping objectives between the projected targets for the active ingredient of TIP-Heal and those related to DFU.

### Selected target genes of DFU-related target for TIP-Heal active ingredient

The selected target genes were standardized in UniProt, a widely used database for protein information (<https://www.uniprot.org>) (22). The organism selected for this standardization process was "Homo sapiens," resulting in a comprehensive list of target genes relevant to DFU in humans.

### Gene ontology (GO) and Kyoto Encyclopaedia of Gene and Genomes (KEGG) analysis of crucial protein target

The species under consideration was homo sapiens, and the threshold for determining significant differences was set at  $P < 0.05$ . The identified critical targets were uploaded

to the DAVID (Database for annotation, visualization, and integrated discovery) platform for conducting GO and KEGG pathway enrichment analysis. The DAVID platform was accessed at <http://david.ncifcrf.gov/>, while the KEGG pathway was obtained at <http://www.genome.jp/kegg/>. GO function enrichment analysis included the examination of molecular function (MF), cellular component (CC), and biological process (BP). The results were plotted and shown using an online bioinformatics platform designed for data processing and visualization, accessible at <http://bioinformatics.com.cn>.

#### Protein-protein interaction (PPI) network construction

PPI networks and functional enrichment analysis were carried out on previously described common targets by applying STRINGdb (Search Tool for the Retrieval of Interacting Genes/Proteins) (<https://string.db.org>). The data obtained were then brought into Cytoscape 3.10.1 (23) to create PPI, analyze essential regulator proteins in PPI, and identify crucial proteins with the highest degree of topological analysis. Furthermore, the cytoHubba plugin in Cytoscape aimed to investigate critical nodes/hubs in the PPI network. The determination of the top five crucial proteins was based on the criteria 'betweenness', 'degree', and 'closeness', where the data could be found in the Cytoscape application (24).

#### Molecular docking of primary active ingredients to a critical protein target

The ability and affinity of TIP-Heal components to the selected vital protein targets were assessed using molecular docking. The Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank

(PDB, <https://www.rcsb.org/>) provided the 3D structures of the target protein. Subsequently, MOE15.10 was used to carry out molecular docking and obtain the 3D structures of the active components (25), with  $-5.0$  kcal/mol binding energy as the chosen criteria (26). In summary, the work scheme in this research is shown in Figure 1.

## Results

### Identification of TIP-Heal bioactive compounds and their respective target in human cells

A total of 20 active compounds were obtained for TIP-Heal from the CMUAP database and PubMed. The compounds with their chemical structure are shown in Figure 2. Subsequently, 417 possible protein targets were discovered in the 20 bioactive chemicals, sourced from the Swiss Target Prediction database (see Table S1 of Supplementary file 1). The 20 bioactive chemicals from 3 plants are as follows.

### Identification of DFU-related targets for TIP-Heal active ingredients

A total of 3016 protein targets were discovered for DFU, of which 2980 proteins were obtained from the GeneCard database, 107 from DisGeNET, and 20 from the therapeutic target database. A comprehensive collection of proteins was assembled and subsequently selected to mitigate the occurrence of redundant data. An intersection was performed between DFU target and metabolite target proteins derived from Swiss targets (see Figure S1). A total of 191 protein targets were found to be common between the active chemical targets and those linked to DFU. The 20 active components of TIP-Heal extract were strongly interconnected.

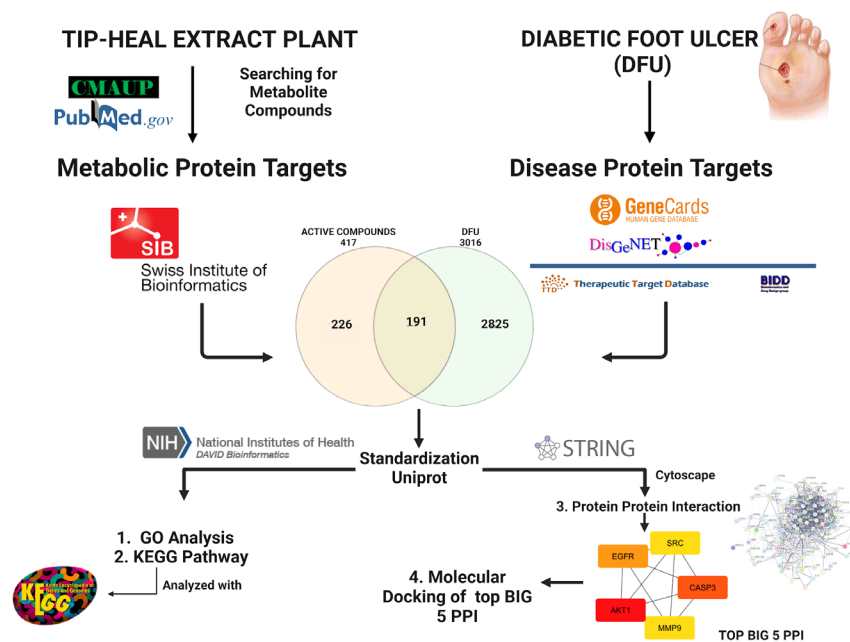
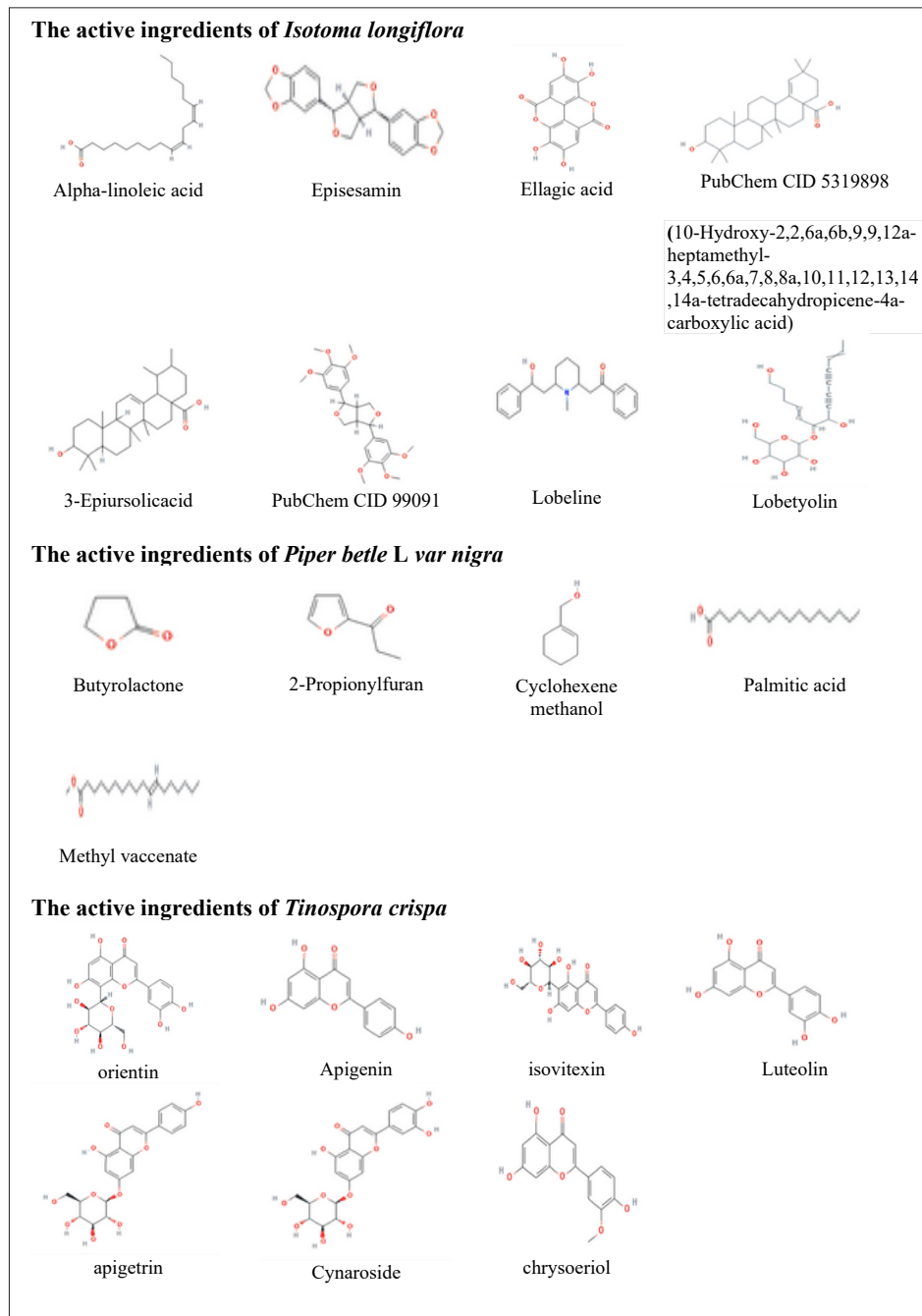


Figure 1. Schematic illustration of network pharmacology study of TIP-Heal extract for the treatment of diabetic foot ulcers.



**Figure 2.** Active ingredients in *Tinospora crispa*, *Isotoma longiflora*, and *Piper betle L var nigra* (TIP-Heal extract).

### Standardisation of DFU-related target for TIP-Heal active ingredients

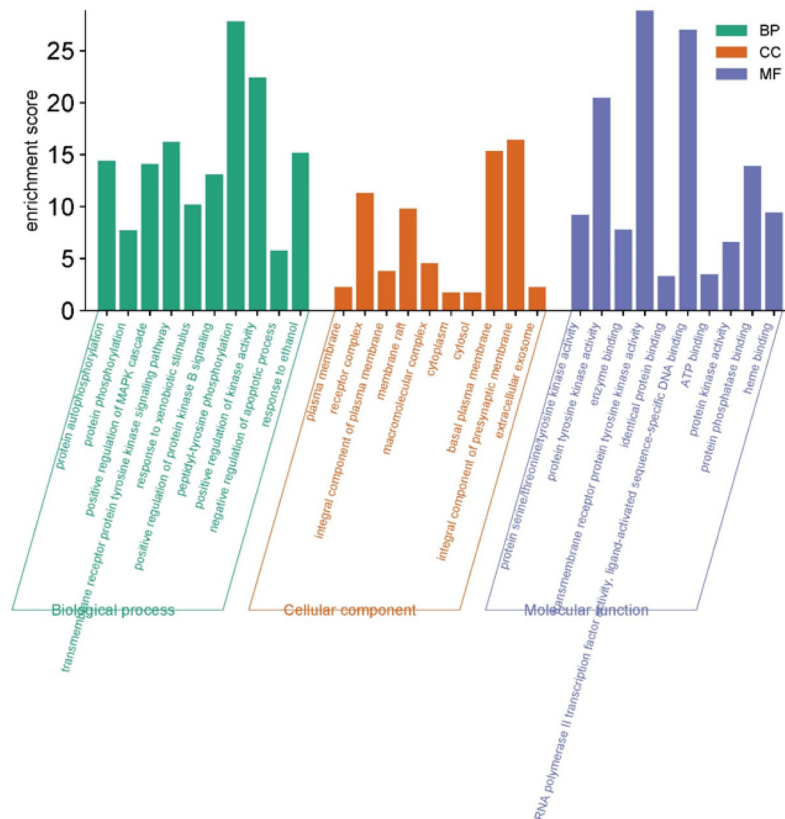
A total of 93 proteins with active sites were identified by UniProt standardizing those associated with human beings through a keyword search using the term “homo sapiens”. The proteins contained in TIP-Heal and their effect on DFU with human subjects are displayed in Table S1 (See [Supplementary file 1](#)).

### Exploration of GO and KEGG for enrichment

The DAVID database was applied to carry out a Gene Ontology enrichment study, aiming to elucidate the

biological functions associated with a set of 93 frequently identified targets, using a significance level of  $P < 0.05$ . Seven hundred thirty-eight enrichment items were acquired for the BP category, 109 for the CC, and 175 for the MF. The top 10 enrichment items within each GO category, arranged in ascending order based on their  $P$  values, enrichment score, count, and rich factor (%) are shown in [Figure 3](#).

The enrichment analysis of signaling pathways was conducted for 93 frequently targeted proteins by applying the KEGG pathway database. This analysis aimed to identify the critical signaling pathways associated with



**Figure 3.** The gene ontology (GO) diagram of TIP-Heal extract to heal diabetic foot ulcer (DFU) based on biological processes (BP), cellular components (CC), and molecular function (MF). The genes from TIP-Heal extract that influenced the healing of diabetic foot wounds were determined based on biological processes (seen on the green diagram), cellular components (seen on the orange diagram), and molecular function (seen on the blue diagram). The top ten enrichment items are those obtained based on  $P$  values, enrichment score, count, and rich factor (%). TIP-Heal: *Tinospora crispa*, *Isotoma longiflora*, and *Piper betle* L var *nigra* extracts in a ratio of 2:1:1.

DFU affected by the active ingredient in TIP-Heal extract. A total of 159 ones were discovered in this study, and the top most significant 20 ones, according to the  $-\log_{10}$  ( $P$  value), enrichment score, and count, are show in Figure 4. The KEGG-based pathway analysis proved that endocrine resistance was important in regulating this biological process. The diagram below illustrates the process of endocrine resistance.

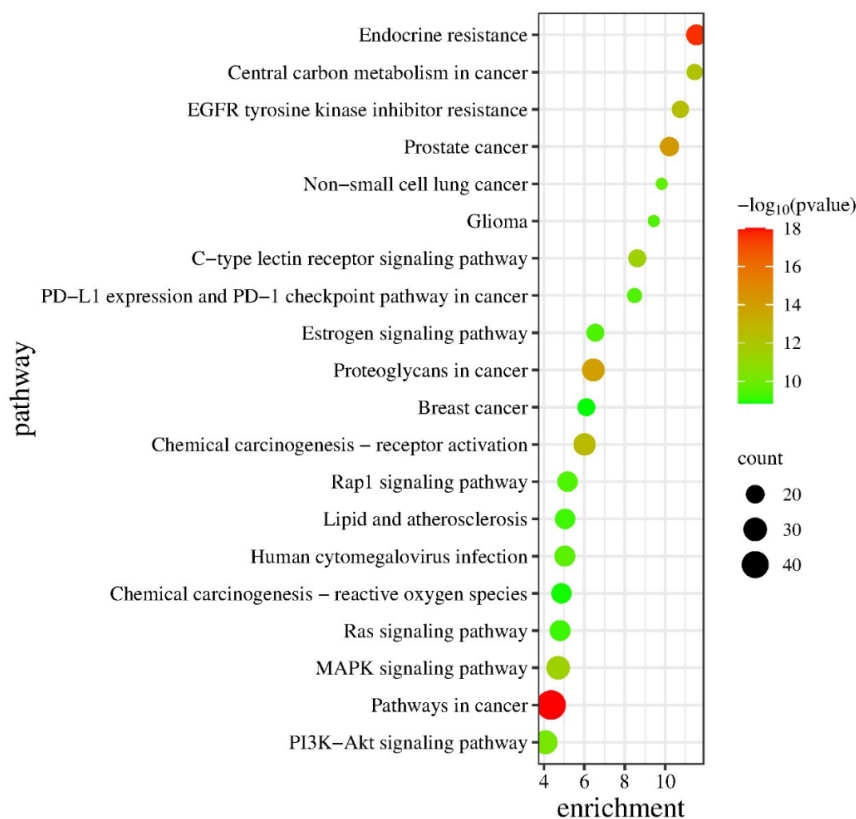
**PPI network creation referring to the construction of PPIs**  
A PPI network was created for the targets discovered by applying the STRING database (27). A total of 93 targets could interact with other proteins, resulting in the observation of 830 edges within the network. The average degree of each protein was found to be 17.8, while the average local clustering coefficient was 0.556. Furthermore, the predicted edge was 327, and the  $P$  value was found to be less than  $1.0 \times 10^{-16}$ . The relationship data regarding each node is presented in Figure S2.

The top five proteins in the PPI network, as determined by Cytohubba in Cytoscape, showed the highest degree. It was observed that these five proteins might serve as the principal hubs responsible for the wound-healing activity of TIP-Heal in the context of DFU. These include

AKT1, CASP3, EGFR, SRC, and MMP-9. PPI analysis for the 93 shared targets between TIP-Heal extract bioactive chemical and the DFU is described in Table S1.

#### Molecular docking of the bioactive ingredient of TIP-Heal with the five focus proteins identified in PPI

The PPI, as mentioned above, identified five significant protein targets. These protein targets presumably serve as crucial biological hubs responsible for the healing activity of TIP-Heal in the context of DFU. A series of docking analyses were carried out to investigate the potential direct binding of the active component to the hub proteins. Specifically, the binding interactions between the 20 bioactive ingredients and the five focus targets were examined, using a selection cutoff of  $-5.0$  kcal/mol for the binding energy. The presence of a critical energy value that was less than zero denoted that the ligand was capable of directly attaching to the protein receptor. The low-level binding energy value indicates that there is a stronger affinity between the two entities. A ligand molecule is considered to have a favorable binding affinity when its value is less than  $-5.0$  kcal/mol (28). Molecular docking analysis results showed that four pairs of ingredients and targets had binding energies that met the criteria.



**Figure 4.** The Kyoto encyclopaedia of gene and genomes (KEGG) pathway of TIP-Heal in diabetic foot ulcer (DFU). The KEGG pathway analysis revealed the top twenty signaling pathways were significantly enriched and controlled by the active ingredient of TIP-Heal extract in DFUs. The dot's color represents the range of P values, enrichment score, and count, while its size and color got redder and more located to the right, corresponding to the number of proteins linked to the identified pathway. TIP-Heal: *Tinospora crispa*, *Isotoma longiflora*, and *Piper betle* L var *nigra* extracts in a ratio of 2:1:1.

This suggests that the active ingredients, specifically alpha-linoleic acid, 3-epiursolic acid, palmitic acid, and PubChem CID 5319898, have a strong affinity for the four hub protein targets: AKT1, CASP3, EGFR, and SRC. However, no substantial binding was observed between the active components of TIP-Heal and the MMP-9 protein, as shown in Table 1.

The active ingredient PubChem CID 5319898 can bind to four central focus targets, namely AKT1, CASP3, EGFR, and SRC, potentially inducing a synergistic effect across various signaling pathways. These results suggested that an active ingredient with a code PubChem CID 5319898 could be a viable candidate for treating DFU. Furthermore, this study provides valuable data support for the design of future experimental investigations in this area. The visualisation of the bond between the active ingredients and protein, which binds amino acid residues in detail is shown in Figure 5.

## Discussion

DFU is widely recognized as a significant chronic consequence of diabetes, contributing significantly to both disability and mortality rates (29). Meanwhile, TIP-Heal is a polyherbal formulation consisting of three herbal extracts, namely *T. crispa*, *I. longiflora*, and *P.*

*betle* L var *nigra*, in a ratio of 2:1:1. This combination is a potential treatment for DFU with wound healing properties. A total of 20 active chemicals were identified through screening in TIP-Heal extract, including a group of fatty acids (alpha-linoleic acid, palmitic acid, methyl vaccinate), steroids (episesamin, PubChem CID 5319898, 3epiursolic acid, PubChem 99091), furan (butyrolactone, 2-propionylfuran), an alkaloid (lobeline), flavonoids (orientin, apigenin, isovitexin, luteolin, apigenin, cymaroside, chrysoeriol), and phenolics (ellagic acid, lobetyolin). Phenolic groups potentially inhibit carbohydrate hydrolyzing enzymes and have anti-diabetic, anti-inflammatory, and anti-infection potential (30). Flavonoids were discovered to have a beneficial impact on treating diabetic wounds by controlling various pathways such as MMP-2, MMP-8, MMP-9, MMP-13, Ras/Raf/MEK/ERK, PI3K/Akt, and nitric oxide. Flavonoids may have the potential to be used as a treatment to reduce the harmful consequences of diabetic wounds (31).

The diagram of GO analysis revealed that peptidyl tyrosine phosphorylation and kinase activity positive regulation significantly influenced the biological process underlying DFU treatment. Ligands interacting to the EGF receptor (EGFR) induce receptor dimerization and autophosphorylation, leading to the tyrosine

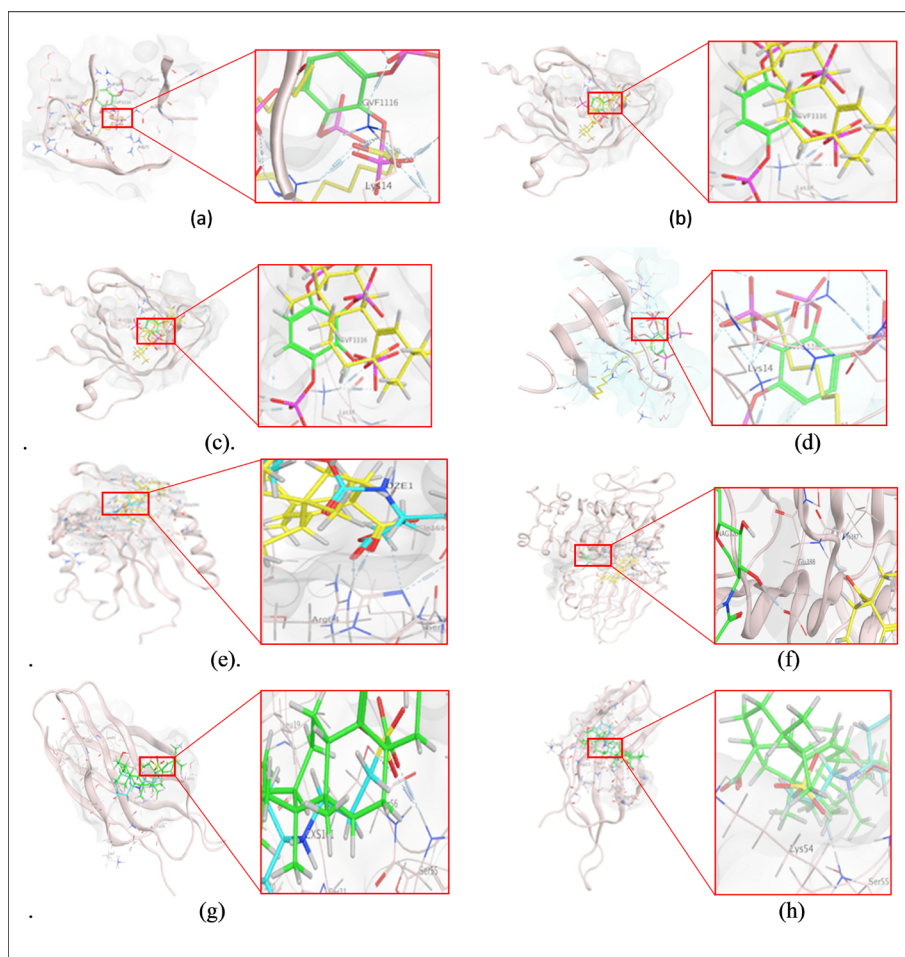
**Table 1.** The binding energy of TIP-Heal extract active ingredient to key protein targets

Hub protein target	PDB IDs	Active ingredient	Plant	Energy binding (kcal/mol)	Residue interaction	Type of interaction	Distance
AKT1	2UVM	Alpha-linoleic acid	<i>Isotoma longiflora</i> L	-11.2	O(51)-NZ.LYS14	H acceptor	2.94
	2UVM	PubChem CID 5319898	<i>Isotoma longiflora</i> L	-15.4	O(75)-NZ.LYS14	H acceptor	2.93
	2UVM	3-Epiursolicacid	<i>Isotoma longiflora</i> L	-11.3	O(79)-NZ.LYS14	H acceptor	2.92
	2UVM	Palmitic acid	<i>Piper betle</i> L var <i>nigra</i>	-12.3	O(49)-NZ.LYS14	H acceptor	3.01
CASP3	3GJR	PubChem CID 5319898	<i>Isotoma longiflora</i> L	-8.4	O(75)-NE.ARG64	H acceptor	2.99
EGFR	5WB7	PubChem CID 5319898	<i>Isotoma longiflora</i> L	-5.1	O(76)-N.Glu 388	H acceptor	3.07
SRC	5MTJ	PubChem CID 5319898	<i>Isotoma longiflora</i> L	-7.6	O(76)-N.SER 55	H acceptor	3.00

PDB IDs, Protein database identities.

phosphorylation of intracellular proteins. A research conducted in a controlled environment showed that the activation of EGFR promoted the process of re-epithelialization by enhancing the proliferation and movement of keratinocytes in wounds (32). This process was primarily occurred within the integral component

of the presynaptic and basal plasma membrane. It was characterized by the MF of transmembrane receptor protein tyrosine kinase activity, ribonucleic acid (RNA) polymerase II transcription factor activity, and ligand-activated sequence-specific deoxyribonucleic acid (DNA) binding. Receptor tyrosine kinases are integral to various



**Figure 5.** Identification of eight strong interactions between four TIP-Heal bioactive compounds and their respective protein targets through docking analysis. These interactions include a) AKT and alpha linoleic acid, b) AKT1 and PubChem CID 5319898, c) AKT1 and 3-Epiursolicacid, d) AKT1 and palmitic acid, e) CASP3 and PubChem CID 5319898, f) EGFR and PubChem CID 5319898, g) SRC and PubChem CID 5319898, and h) SRC and 3-Epiursolicacid. AKT1: Serine/Threonine Kinase1; CASP3: Caspase-3; EGFR: Epidermal growth factor receptor; SRC: Proto-oncogene tyrosine-protein kinase Src. TIP-Heal: *Tinospora crispa*, *Isotoma longiflora*, and *Piper betle* L var *nigra* extracts in a ratio of 2:1:1.

cellular activities, including metabolic regulation, cell-cycle modulation, cell viability, cellular replication, movement, and specialization (33).

In the diagram of KEGG pathway analysis, enrichment refers to the count of all genes (proteins) that are annotated within a particular pathway. Figure 4 illustrates that the most significant enrichment is related to endocrine resistance. The pathway related to cancer and endocrine resistance had a lower margin of error (*P* value), whereas the pathway associated with cancer enrichment was rather small. The count represents the number of genes that exert an influence among all the genes present in the KEGG Pathway.

Examining PPI using the metrics such as degree, betweenness, and closeness identified AKT1, CAPS3, EGFR, SRC, and MMP-9 as those demonstrating the 5 highest degree. These proteins are also closely connected to other nodes, showing their propensity to facilitate the initiation of signaling cascades. AKT, in particular, is important in angiogenesis, which refers to the physiological mechanism responsible for developing and producing novel blood vessels. Angiogenesis is critical in wound healing, development, and tumor formation (34). PI3K/Akt pathway regulates cell proliferation, migration, and survival (34,35). AKT mediates the response to insulin and anti-inflammatory agents (36). Studies have shown increased expression of PI3K, AKT, glycogen synthase kinase-3 beta (GSK3 $\beta$ ), total mammalian target of rapamycin (mTOR), phosphorylated (p)-mTOR, nuclear factor- $\kappa$ B (NF- $\kappa$ B), and caspase 3 proteins related to the PI3K/AKT, NF- $\kappa$ B, and  $\beta$ -catenin pathways in diabetic wounds (37). Caspases play a critical role as mediators of programmed cell death or apoptosis. In particular, caspase-3 (CASP3) is a commonly activated protease associated with programmed cell death, facilitating the selective cleavage of numerous vital cellular proteins (38). The impaired wounds healing in individuals with diabetes is often accompanied by an increase in caspase-3, -8, and -9 activity as well as a substantial decrease in transforming growth factor  $\beta$  and VEGF expression. These changes result in a diminished ability of the wounded tissue to generate new blood vessels and promote angiogenesis due to the impaired mobilization of endothelial progenitor cells (39). In general, the healing process of DFU is intricately associated with various proteins, including AKT1, tumor protein p53 (TP53), IL6, CASP3, TNF, and VEGFA (40).

EGFR is important in cellular differentiation and proliferation when activated by the binding of its ligands. The receptor is situated on the cellular membrane, where the interaction between a ligand and the receptor triggers the activation of a tyrosine kinase within the intracellular domain (41). Ligand-dependent EGFR activation elicits collective cell migration into the wound (42). This tyrosine kinase enzyme phosphorylates specific intracellular substrates, initiating signaling pathways that ultimately result in cellular proliferation (43). According

to previous studies, EGFR promotes cell proliferation, angiogenesis, and invasion (44,45). The motility of keratinocyte stem cells, driven by the EGFR- collagen type XVII  $\alpha$ 1 (COL17A1) axis, plays a crucial role in epidermal regeneration, suggesting a promising therapeutic strategy for addressing defective skin regeneration (46).

The SRC protein kinase significantly influences various cellular processes: cell differentiation, proliferation, and survival. The EGFR stimulation induces cellular proliferation and subsequently triggers the activation of SRC precisely during G2 (second growth phase) transition to the mitosis (M) phase. The protein kinase is important in various cellular processes, including cell adhesion, cell shape and motility, and bone resorption (47). The effects of insulin through the SRC/mitogen-activated protein kinases pathway contribute to vasoconstriction mediated by endothelin-1 (ET-1), induce inflammation through plasminogen activator inhibitor-1 (PAI-1), as well as stimulate the proliferation and migration of vascular smooth muscle cells (48). Moreover, the SRC protein is important in the process of angiogenesis, which is facilitated by advanced glycation end products. This role is mainly mediated through the phosphorylation of extracellular signal-regulated kinases (ERK) by the receptor for advanced glycation end products (RAGE)-SRC-ERK pathway (49). The interplay between c-SRC and phospholipase D in transmodulation may facilitate cellular proliferation by enhancing the activation of mitogenic signaling pathways (50).

The regulation of pathological remodeling processes, characterized by inflammation and fibrosis, is controlled by MMP-9. This enzyme directly degrades proteins inside the ECM while also activating cytokines and chemokines, regulating tissue remodeling (51). The presence of infection leads to an elevation in active MMP-9 levels in individuals with diabetes. Furthermore, MMPs are important in the immunological response to infection. The induction of MMP-2 and MMP-9 expression may occur due to T-cell activation. MMP-9 plays a role in mediating T-cell migration. In inflammation, prostaglandin E2 is important in the upregulation of MMP-9 expression. This upregulation, in turn, leads to the induction of dendritic cell migration, which initiates the immune response (52).

Following an injury, there is an observed elevation in neutrophil numbers in the affected site, facilitating the immune response against potential infections. Neutrophils release reactive oxygen species (ROS), MMP-8, and MMP-9, which have bactericidal properties and contribute to creating a thrombus. This serves as a protective mechanism to prevent excessive blood loss at the site of a wound. The activation of ROS leads to NF- $\kappa$ B upregulation, resulting in the MMP-9 rise, which has a negative impact on the healing process of DFU (53).

The molecular docking process investigated the interaction between four proteins and the active component in TIP-Heal. The docking procedure used in



this study was a rigorously verified process, comprising the production of both ligands and proteins, validation of posture and score, and validated outcomes (54). The results showed that among the compounds examined (PubChem 5319898, 3-epiursolic acid, alpha-linoleic, and palmitic acid), PubChem CID 5319898 had the highest affinity towards the four selected proteins while showing a weaker effect towards the MMP-9 protein. The analysis showed that among the chemicals identified by PubChem CID 5319898, the 3-epiursolic, palmitic, and alpha-linoleic acids had promising characteristics as possible medication candidates for treating DFU healing.

The limitation of this research was the data used for network pharmacology (for screening TIP-Heal and DFUs protein), which was based on an existing database; the results are very dependent on the completeness of the data in the program database used. However, its strength is so that it can predict the synergism of the combination of 3 polyherbal (*T. crista*, *I. longiflora*, *P. betle* L var. *nigra*). This provides basic evidence to explore the efficacy of the extract combination in further study *in vitro* and *in vivo*. The active content of *I. longiflora* (PubChem 5319898, 3-Epiursolic acid, and alpha-linoleic) and *P. betle* L var. *nigra* (palmitic acid) is predicted to be a potent candidate drug for DFU therapy.

### Conclusion

The pharmacological network analysis results showed that four proteins namely AKT1, CAPS3, EGFR, and SRC influenced the process of healing in DFU. Furthermore, molecular docking analysis outcomes showed that the active compounds PubChem CID 5319898, 3-Epiursolic acid, Palmitic acid, Alpha-linoleic acid could be used as biomarkers in in-vivo and in-vitro studies.

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### Authors' contributions

**Investigation:** Zullies Ikawati, Retno Murwanti, Nanang Fakhrudin.

**Methodology:** Happy Elda Murdiana, Zullies Ikawati, Retno Murwanti, Nanang Fakhrudin.

**Project administration:** Zullies Ikawati.

**Resources:** Happy Elda Murdiana, Zullies Ikawati, Retno Murwanti, Nanang Fakhrudin.

**Supervision:** Zullies Ikawati; Retno Murwanti, Nanang Fakhrudin.

**Validation:** Zullies Ikawati.

**Visualization:** Happy Elda Murdiana.

**Writing-original draft:** Happy Elda Murdiana.

**Writing-review & editing:** Zullies Ikawati, Retno Murwanti, Nanang Fakhrudin. All authors read and

approved the final manuscript.

### Conflict of interests

The authors declare no conflict of interest.

### Ethical considerations

This research was done based on the ethics committee approval of the Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine, Public Health and Nursing, Gadjah Mada University DR. Sardjito General Hospital ref. No: KE/FK/1490/EC/2023.

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### Supplementary files

Supplementary file 1 contains Table S1, Figure S1 and Figure S2.

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