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Original Article

SYNERGISTIC POTENTIAL OF *NIGELLA SATIVA* L. AND *TRIGONELLA FOENUM-GRAECUM*: INTEGRATED NETWORK PHARMACOLOGY FOR DIABETIC WOUND HEALING

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ABSTRACT

Objective: Diabetes Mellitus (DM) is a metabolic disorder marked by elevated blood glucose levels, and one of the issues linked to DM involves the development of Diabetic Wounds (DW). DW is susceptible to infection and develops into chronic wounds if not treated properly. This study aimed to investigate the network pharmacology of *N. sativa* L. and *T. foenum-graecum,* emphasizing on their potential as DW treatment candidates.

Methods: Various databases were used in this study, including PubChem, Dr. Duke's phytochemistry and Ethnobotany, and KNApSAcK Family. Swiss Target Prediction and Way2Drug PASS Online were utilized for biological activity and protein target prediction. The DW pathway's protein-protein interactions were examined with the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, Gene Cards, and STRING databases. STRING was used to predict the metabolite's action. The relationship between metabolites and target proteins was predicted using STITCH, and Cytoscape was used to visualize the network.

Result: The results showed that ten active ingredients (five active ingredients in *N. sativa* L. and five active ingredients in *T. foenoem-graecum*) contributed to DW healing by affecting Tumor Necrosis Factor (TNF), Interleukin-1beta (IL1B), JUN, Caspase 3 (CASP3), Interleukin-6 (IL-6), Alpha Kinase Threonine-1 (AKT1), Vascular Endothelial Growth Factor-A (VEGFA), and Mitogen-Activated Protein Kinase 3 (MAPK3) genes. Furthermore, the ten active ingredients correlated with twenty-eight intracellular proteins, resulting in a mechanism involving eight DW signalling pathways.

Conclusion: Based on network pharmacology analysis, we determine that *N. sativa* L. and *T. foenoem-graecum* combination can potentially treat DW.

Keywords: DW, Network pharmacology, *N. sativa* L., *T. foenum-graecum*

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INTRODUCTION

A metabolic disease called Diabetes Mellitus (DM) is characterized by increased blood glucose levels and disturbances in carbohydrate, lipid, and protein metabolism that persist for an extended period. DM can develop due to the body's incapacity to process and control blood glucose because of either an excess of insulin secreted by the pancreas or an incapacity of insulin to control blood glucose levels (Shailaja *et al.*, 2020).

Indonesia is ranked 7th with the highest number of DM sufferers. One of the most severe complications of DM is Diabetic Wounds (DW). Domestic workers are susceptible to infection and will experience impaired wound healing if the wound is not treated correctly from the start [2]; 85% of wounds will develop into chronic wounds and even lead to amputation and death [3, 4]. Impaired DM wound healing is often caused by poor perfusion due to peripheral arterial disease [5]. In addition, impairment can also be caused by a decreased immune system, high oxidation stress, and bacterial resistance to antibiotics [6].

So far, the antiseptic agent often used to treat DW is the *bioplacenton*. *Bioplacenton* contains 10% placenta extract, 0.5% neomycin sulfate, and a gel base [7]. However, if prolonged exposure occurs, *bioplacenton* can cause skin irritation characterized by red spots [8].

Therefore, it is anticipated that one option for treating DW with fewer adverse effects would be the creation of medications from natural materials. Two natural substances that may be used to treat DW are *N. sativa* L. and *T. foenoem-graecum*. *N. sativa* L. has thymoquinone with many pharmacological effects. In diabetic rats, it was demonstrated that applying 20-40% *N. sativa* L. extract will speed up wound healing within 15-18 days [9]. At the same time, *T. foenum-graecum* extract has activity as an antibacterial, such as *Propionibacterium-acne* and *Staphylococcus aureus*, which are often found in DW [10]. Furthermore, commencing on the third day of therapy, a study that combined *N. sativa* L. and *T. foenum-graecum* extracts showed a drop in neutrophil and macrophage counts but an increase in fibroblast and collagen density counts [11].

The one-drug-one-target-one-disease approach is considered less effective in treating DW. Therefore, developing multi-target drugs and network pharmacology has become increasingly popular [12]. Network pharmacology explores how traditional medicines work using in silico models using complex networks composed of "protein-compound/disease-gene". Using the network pharmacology approach, we may look into possible connections between target genes and the active ingredients in the combination of *N. sativa* L. and *T. foenum-graecum*. The network pharmacology approach provides a deeper understanding of drug-target interactions, thus opening new opportunities for exploring more efficient and targeted medicine [13]. This study aimed to investigate the network pharmacology of *N. sativa* L. and *T. foenum-graecum*, focusing on their potential as therapy options for DW, given the background information mentioned above. Hopefully, that this study can serve as a reference for developing safe and effective DW treatments based on target genes.

MATERIALS AND METHODS

This study employed a research method similar to Firzannida *et al.*, (2022). The KNApSAcK family database (http://www.knapsackfamily.com/KNApSAcK/) and Dr. Duke's Phytochemical and Ethnobotanical database (https://phytochem.nal.usda.gov/) provided the metabolites used in this work. Metabolite chemical structures were verified by the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

Swiss Target Prediction platform (http://www.swisstargetprediction.ch/) was used to predict protein targets of the combination metabolites in DW, while the Way2Drug PASS Online platform (https://www.way2drug.com/passonline/)

was used to predict active compounds activity. All targets were restricted to humans.

The KEGG pathway (https://www.genome.jp/kegg/pathway.html), GeneCards (https://www.genecards.org/), and STRING database (https://string-db.org/) were used to evaluate the interaction between proteins that involved in the pathway of the DW. To analyze protein-metabolite interactions related to the DW pathway, the STITCH database (http://stitch.embl.de/) was used.

Using Cytoscape v3.10.1, the pharmacology network of the combination was displayed.

RESULTS AND DISCUSSION

Data regarding the connections between metabolites, biological activities, and species are available in the extensive database KNApSAcK [15]. The phytochemical and ethnobotanical databases maintained by Dr. Duke the freely accessible databases that are highly significant for ethnomedicinal data [16]. The seed oil of *N. sativa* L. contained thirteen metabolites, while the seed components contained eleven. *T. foenum-graecum* reveals twenty-three metabolites in the seed parts, eight metabolites in the plant parts, one metabolite in the fruit, and one in the stem (table 2).

Table 1: Prediction of secondary metabolites in *N. sativa* L

CAS ID	Metabolite	Molecular formula	Plant part
60-33-3	Linoleic acid	C18H32O2	Seed Oil
112-80-1	Oleic acid	C18H34O2	Seed oil
57-88-5	Cholesterol	C27H46O	Seed oil
469-38-5	Cycloartenol	C30H50O	Seed oil
481-25-4	Lophenol	C28H48O	Seed oil
559-70-6	beta-Amyrin	C30H50O	Seed oil
127-22-0	Taraxerol	C30H50O	Seed oil
117-39-5	Quercetin	C15H10O7	Seed
469-39-6	Cycloeucalenol	C30H50O	Seed oil
16910-32-0	Obtusifoliol	C30H50O	Seed oil
490-91-5	Thymoquinone	C10H12O2	Seed oil
472-28-6	(-)-Butyrospermol	C30H50O	Seed oil
197250-98-9	Kaempferol 3-glucosyl-(1->2)-galactosyl-(1->2)-glucoside	C33H40O21	Seed
197250-97-8	Quercetin 3-glucosyl-(1->2)-galactosyl-(1->2)-glucoside	C33H40O22	Seed
197294-29-4	Quercetin 3-(6''''-feruloylglucosyl)-(1->2)-galactosyl-(1->2)-glucoside	C43H48O25	Seed
98063-20-8	Nigellicine	C13H14N2O3	Seed
120993-86-4	Nigellidine	C18H18N2O2	Seed
2/9/4594	Nigellimine	C12H13NO2	Seed
99-49-0	Carvone	C10H14O2	Seed
117652-9	Gramisterol	C29H48O	Seed oil
96562-85-5	Nigellimine N-oxide	C12H13NO3	Seed
39461-20-6	Nigellone	C18H22O4	Seed
514-46-5	Tirucallol	C30H50O	Seed oil

Notes: CAS ID (Chemical Abstracts Service Registry Number) is a unique identification number assigned to each chemical substance recognized globally.

Table 2: Prediction of secondary metabolites in *T. foenum-graecum*

CAS ID	Metabolite	Molecular formula	Plant part
59-43-8	Thiamine	C12H17N4OS	Seed
578-74-5	Apigenin 7-O-beta-D-glucopyranoside	C21H20O10	Plant
4261-42-1	Isoorientin	C21H20O11	Seed
38953-85-4	Isovitexin	C21H20O10	Seed
28608-75-5	Orientin	C21H20O11	Seed
3681-93-4	Vitexin	C21H20O10	Seed
71-00-1	L-Histidine	C6H9N3O2	Seed
535-83-1	Trigonelline	C7H7NO2	Seed
485-72-3	Formononetin	C16H12O4	Plant
511-96-6	Gitogenin	C27H44O4	Seed
126-19-2	Sarsasapogenin	C27H44O3	Seed
126-18-1	Smilagenin	C27H44O3	Seed
77-60-1	Tigogenin	C27H44O3	Seed
512-06-1	Yamogenin	C27H42O3	Seed
2150-11-0	3',4',7-Trihydroxyflavone	C15H10O5	Plant
25615-14-9	Kaempferol 3,7-di-O-glucoside	C27H30O16	Plant
35927-38-9	Vicenin 1	C26H28O14	Plant
23666-13-9	Vicenin	C27H30O15	Seed
59282-55-2	Vitexin 2''-O-p-coumarate	C30H26O12	Seed
382141-37-9	Kaempferol 3-glucosyl-(1->2)-(6''-acetylgalactoside)-7-glucoside	C35H42O22	Plant
382143-42-2	Quercetin 3-glucosyl-(1->2)-galactoside-7-glucoside	C33H40O22	Plant
14144-06-0	Diosgenin 3-O-beta-D-glucopyranoside	C33H52O8	Seed
470-01-9	Neotigogenin	C27H44O3	Seed
728881-06-9	Saponin SA-III	C38H60O12	Fruit
14002-93-8	3,4,7-Trimethylcoumarin	C12H12O2	Stem
99664-39-8	Trigofoenoside D	C51H84O23	Seed
99705-66-5	Trigofoenoside A	C45H74O18	Seed
99753-11-4	Trigofoenoside B	C45H76O19	Seed
99753-12-5	Trigofoenoside C	C51H86O23	Seed
511-97-7	Yuccagenin	C27H42O4	Seed
6811-13-8	Neogitogenin	C27H44O4	Plant
94714-56-4	Trigofoenoside F	C51H84O23	Seed
94714-57-5	Trigofoenoside G	C56H92O27	Seed
959774-34-6	Apigenin 6-C-beta-arabinopyranosyl-8-C-beta-galactopyranoside	C26H28O14	Plant

In traditional medical practices, *N. sativa* L. has a long history, particularly in Asian nations like Indonesia. It has been shown that *N. sativa* L.'s anti-inflammatory, antibacterial, tissue growth-stimulating and antioxidant properties are therapeutically beneficial for skin wound healing [17]. *T. foenum-graecum*, popularly known as fenugreek, is a small perennial plant in the *Fabaceae* family used as a medication for several diseases, and an antioxidant [18], and an anti-inflammatory [19]. When applied to a wound, its anti-inflammatory properties are released, aiding healing process and reducing inflammation. Furthermore, fatty acids found in the seeds help to form collagen, which promotes faster wound healing and preserves skin suppleness. Prediction of biological activity and protein targets. A metabolite's biological potential or bioactivity of a metabolite can be predicted through Way2Drug PASS Online**,** which is accompanied by a probability of activity (Pa) value. Based on the structure of both new and existing organic compounds, Way2Drug PASS Online provides more than 4000 categories of biological activity with an average accuracy of 95%. Using Way2Drug PASS Online in the early stages of the study makes it possible to select unpromising compounds [20]. The combination of active chemicals' predicted bioactivity is shown in (table 3).

Table 3: The prediction of bioactivity of active compounds in the combination of *N. sativa* L. and *T. foenum-graecum*

Main compound	Bioactivity prediction	Pa* (Probability of Activity)	Pi (Probability of Inactive)
Thymoquinone	Antiinflammatory	0.601	0.031
(CID 10281)	Antibacterial	0.353	0.042
Quercetin	Antiinflammatory	0.689	0.017
(CID 5280343)	Antibacterial	0.387	0.033
Beta-amyrin	Antiinflammatory	0.843	0.005
(CID 73145)	Antibacterial	0.206	0.112
Oleic Acid	Antiinflammatory	0.614	0.029
(CID 445639)	Antibacterial	0.332	0.048
Linoleic Acid	Antiinflammatory	0.730	0.012
(CID 5280450)	Antibacterial	0.335	0.047
Diosgenin	Antiinflammatory	0.766	0.009
(CID 99474)	Antibacterial	0.396	0.031
Tigogenin	Antiinflammatory	0.714	0.014
(CID 99516)	Antibacterial	0.460	0.020
Yuccagenin	Antiinflammatory	0.820	0.005
(CID 3083608)	Antibacterial	0.393	0.032
Smilagenin	Antiinflammatory	0.714	0.014
(CID 91439)	Antibacterial	0.460	0.020
Gitogenin	Antiinflammatory	0.790	0.008
(CID 441887)	Antibacterial	0.456	0.021

Notes: Pa (Probability of Activity) and Pi (Probability of Inactive) PASS prediction results are interpreted: (i) only activities with Pa>Pi are possible for a given compound. (ii) If Pa>0.7, there is a high probability of finding high activity experimentally.

All metabolites in *N. sativa* L. have biological potential associated with DW through anti-inflammatory and antibacterial activities. The inflammatory activity of *N. sativa* L. is predicted due to the content of thymoquinone, quercetin, and oleic acid compounds. The three active compounds have biological potential related to DW and similarities with medicinal compounds, with Pa values<0.7. In antibacterial activity, all test compounds have a Pa value<0.5, which means that these compounds have low biological activity. The predicted bioactivity of active compounds from *T. foenum-graecum* indicates that all compounds exhibit anti-inflammatory activity and are similar to known drug compounds (Pa>0.7). However, regarding antibacterial bioactivity, all test compounds show a Pa value<0.5, signifying low biological activity in both in silico and laboratory-scale tests. Despite this, there is a promising potential for discovering new chemical entities [21].

The metabolite content of the combination can target a wide variety of protein classes in the body, including those relevant to DW, according to the target protein prediction database of the Swiss Target Prediction. Probability values>0 were obtained for proteins evaluated using the GeneCards database (table 4). From 0 to 1, this number shows how comparable the forecast results are. The likelihood of an accurate forecast increases with probability [22]. GeneCards is a human gene database containing known and successfully predicted genomic, proteomic, transcriptomic, genetic, and functional data. The investigation identified one hundred thirty-one target proteins involving *N. sativa L.* and *T. foenum-graecum* metabolites in DW were identified from the investigation.

Table 4: Prediction of metabolites target proteins in the combination of *N. sativa* L. and *T. foenum-graecum*

PubChem ID	Compound name	Target genes linked to DW
10281	Thymoquinone	TLR9, NOS3, IL6, PTPN1
5280343	Quercetin	KDR, MMP9
73145	Beta amyrin	PPARG, MAPK3, PTPN1, NOS2, MDM2
445639	Oleic Acid	PPARG, NOS2, MAPK3, PTPN1
5280450	Linoleic Acid	PPARG, NOS2, IL6, MAPK14, MAPK1, MDM2, MAPK3
99474	Diosgenin	PTPN1, MDM2, MAPK14
99516	Tigogenin	MDM2, NOS2, MAPK14, VEGFA, FGF2, KDR
3083608	Yuccagenin	MAPK14, MDM2, PTPN1
91439	Smilagenin	KDR, MAPK14, MDM2, VEGFA, NOS2
441887	Gitogenin	KDR, MAPK14, MDM2, MAPK1, FGF2, VEGFA

The KEGG pathway provided the DW signaling pathway prediction. Target protein interactions having a minimum interaction score of>0.400 (medium confidence) within a range of 0-1, as determined by STRING, were the focus of the study. Interactions are more physiologically important, and the higher the interaction score. This number denotes a biologically significant link (interaction). With 16.568 chemicals in its database, KEGG is a metabolic pathway that may be used to predict an organism's metabolic pathways [23].

Researchers frequently utilize STRING to evaluate protein-protein interactions in bioinformatics studies because of its high coverage, simplicity of usage, and reliable scoring system [24]. Eight target proteins were generated by analyzing DW pathway prediction using the KEGG pathway and STRING. These target proteins included the TNF signaling pathway, the Advanced Glycation End Products (AGE) receptor AGE (RAGE) signaling pathway in diabetic complications, the Toll-Like Receptor (TLR) signaling pathway, the NF-kappa B (NFKB) signaling pathway, the Vascular Endothelial Growth Factor (VEGF) signaling pathway, the Mitogen-Activated Protein Kinase (MAPK) signaling pathway, and the Transforming Growth Factor Beta (TGF-β) signaling pathway (table 5).

Table 5: Predicted relationship in the combination of *N. sativa* L. and *T. foenum-graecum* target proteins with DW signaling pathways

Proteins involved in DW	DW signaling pathway	Proteins involved in DW signaling pathways
TNF	IL17 signaling pathway	IL6, CASP3, IL1B, TNF, MAPK3, JUN, VEGFA
IL1B	TNF signaling pathway	IL6, CASP3, IL1B, TNF, MAPK3, JUN, AKT1
JUN	AGE-RAGE signaling pathway in diabetic complication	IL6, IL1B, CASP3, TNF, AKT1, MAPK3, JUN
CASP3	TLR signaling pathway	IL6, IL1B, TNF, AKT1, MAPK3, JUN
IL-6	Nf-kappa B signaling pathway	IL1B, TNF
AKT1	VEGF signaling pathway	AKT1, MAPK3
VEGFA	MAPK signaling pathway	IL1B, CASP3, TNF, AKT1, MAPK3, JUN
MAPK3	TGF-beta signaling pathway	TNF, MAPK3, VEGFA

STITCH's database and analytical tool, called STITCH examines how bioactive compounds interact with the target protein. Fig. 1 depicts the development of the STITCH-based interaction network between metabolite chemicals in *N. sativa* L. and *T. foenum-graecum* and their target proteins. A medium level of confidence is indicated by STITCH results, with a minimum interaction score of>0.400 (medium confidence). The stronger the link between the bioactive molecule and its target protein, the thicker the interaction line. The STITCH interaction analysis indicates that five active substances: diosgenin, linoleic acid, oleic acid, thymoquinone, and quercetin interact directly with surface receptor proteins. Additionally, as a result of integration with STITCH, a network view is available to view the binding affinity of chemical compounds in an interaction network. STITCH briefly explains how a molecule could affect its interaction partner [25]. Moreover, the quantity of intracellular proteins on that surface signifies the transition from chemical signals (extracellular) to intracellular signals.

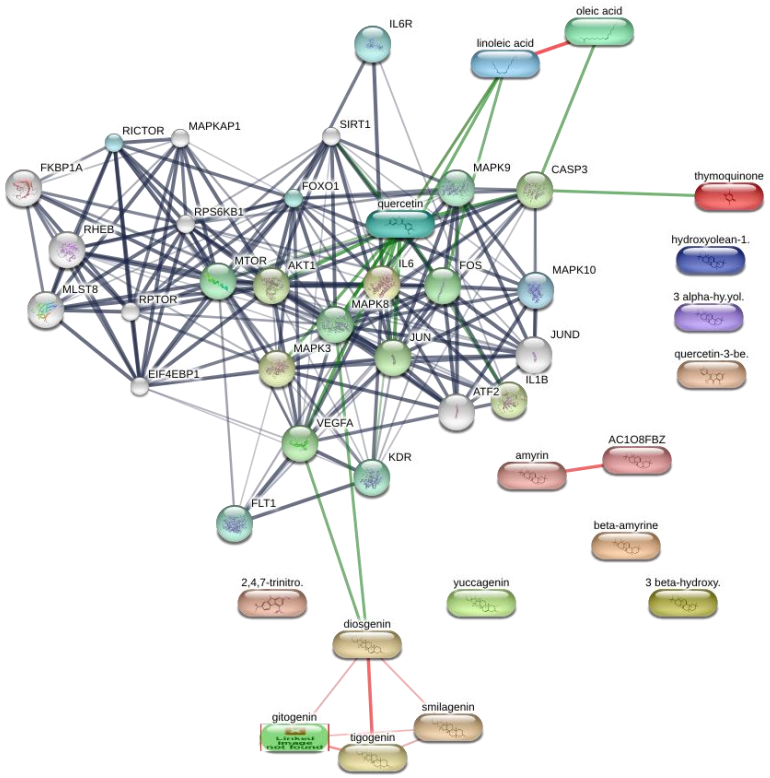

Fig. 1: Confidence results through the STITCH database. Interaction of metabolites and protein intracellular with associated activity. Grey color indicates protein-protein interactions, green indicates chemical-protein interactions, and red indicates metabolite interactions.

Network pharmacology visualized with Cytoscape is shown in fig. 2. Cytoscape visualizes network molecular interactions and biological signaling pathways [26]. *N. sativa* L. and *T. foenum-graecum* metabolites interact with eight surface receptor proteins and twenty-eight intracellular outer proteins. The mechanism involves 8 DW signalling pathways: Interleukin-17 (IL-17), TNF, AGE-RAGE, TLR, NFKB, VEGF, MAPK, and TGF-β.

IL-17 signaling pathway as a proinflammatory cytokine produced by Th 17 will stimulate T cells to secrete proinflammatory mediators such as Interleukin-1 (IL-1), Tumor Necrosis Factor-α (TNF-α), chemokines, and IL-6 [27]. It is known that linoleic acid and quercetin compounds inhibit IL-17 by reducing IL-6 expression, as shown by scores of 0.839 and 0.951, respectively [28, 29]. A family member of IL-17A, commonly referred to as IL-17, has recently been

successfully studied by researchers, and it was found that IL-17-deficient mice had delayed wound closure. Another aspect of wound healing is the repair of blood vessels to provide nutrients to the recovering organs, which often requires the process of angiogenesis (formation of new blood vessels). IL-17 promotes the production of Vascular Endothelial Growth Factor A (VEGFA) by epithelial and fibroblastic cells to stimulate angiogenesis [30]. As demonstrated by interaction values of 0.800 and 0.947, diosgenin and quercetin are hypothesized to transport chemical signals that the IL-17 receptor protein receives, triggering the VEGFA signaling pathway (table 6).

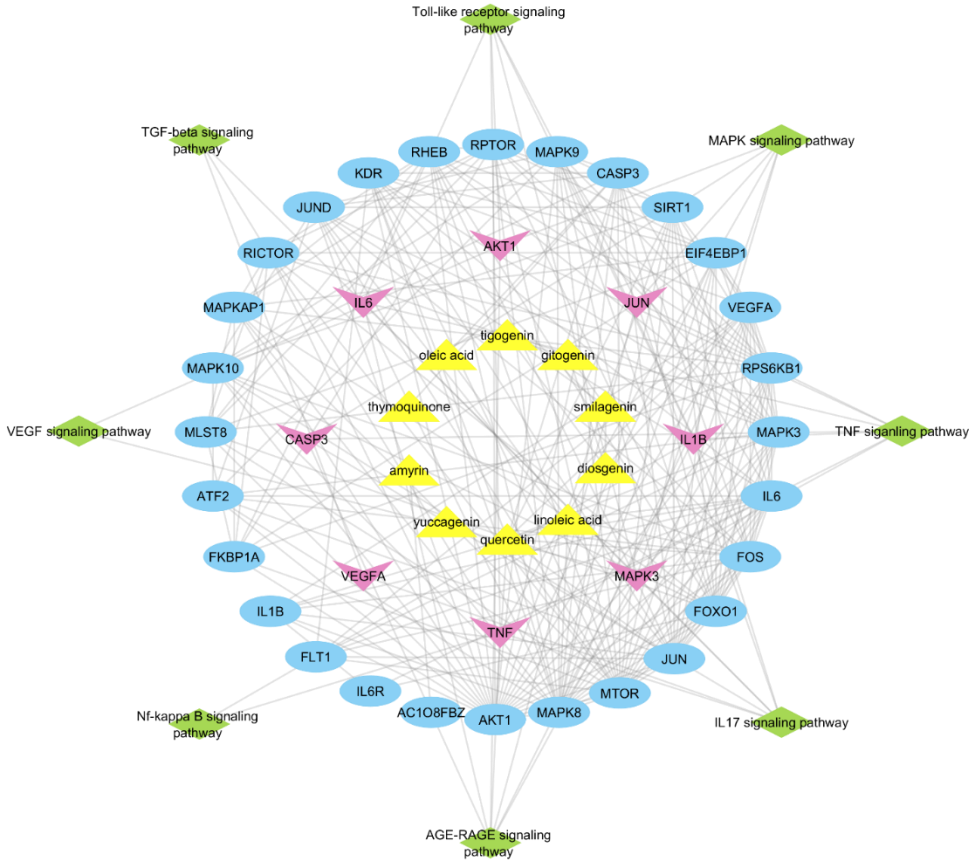

Fig. 2: Visualization of the pharmacological network of metabolites included in the combination of *T. foenum-graecum* and *N. sativa* L. (yellow), surface receptors (purple), and intracellular proteins (blue), and DW signaling pathways (green) with cytoscape

Table 6: Interaction analysis of target proteins and metabolites in the combination of *N. sativa* L. and *T. foenum-graecum*

Node 1	Node 2	Score
Diosgenin	VEGFA	0.800
Linoleic acid	IL-6	0.839
Oleic acid	CASP3	0.826
Quercetin	AKT1	0.957
Quercetin	CASP3	0.953
Quercetin	IL1B	0.738
Quercetin	IL-6	0.951

Monocytes and macrophages can produce TNF-α, which is a proinflammatory cytokine. TNF-α plays a role in many processes in the body, including the pathogenesis of various diseases. TNF-α levels were found to be three times gater in diabetic rat wounds compared to normal wounds. *In vitro* research revealed that TNF-α boosted the expression of apoptosis-related genes, such as Akt and p53, and genes implicated in inflammation, cytokines, TLR, NF-κB, and Casp3 [31]. The compounds oleic acid, quercetin, and thymoquinone are thought to inhibit TNF-α expression through decreasing caspase-3 because they have interaction values of 0.826, 0.953, and 0.822, respectively. Decreased in caspase-3 levels reduce fibroblast apoptosis and increase ECM production [32–34]. In addition, quercetin compounds can inhibit the activation of cJun-NH2 terminal kinase (JNK), as shown by a score of 0.948. Inhibition of JNK activation can reduce the expression of inflammatory genes and protein secretion [35].

Type 2 diabetes and its consequences pathophysiology are linked to gene transcription changes triggered by the AGE-RAGE signaling pathway[36]. Low-density lipoprotein (LDL) oxidation and elevated oxidative stress are essential factors in Cardiovascular Disease (CVD) pathophysiology and are mediated by AGE-RAGE. As a ligand for RAGE, oxidized LDL stimulates the production of TGF-β, C - C-reactive protein (CRP), inflammatory cytokines, and Protein Kinase C (PKC). It activates several intracellular pathways, including NF-κB, p38, MAPK, and JNK. This contributes to the pathogenesis of CVD by promoting vascular calcification and hardening of the blood vessel's medial layer. In addition, under conditions of high glucose levels, various inflammatory factors, such as IL-6, TNF-α, and the AGE-RAGE combination, can activate IL-1β [37]. The quercetin compound is thought to inhibit the AGE-RAGE signaling pathway so inflammatory factors such as IL-1β are not activated, as indicated by an interaction score of 0.738 [38]. Thus, these findings may suggest

that inhibiting AGE-RAGE signaling in DW is connected with a decrease in persistent inflammation and healing success [31].

One of the targeted Pattern Recognition Receptors (PRRs) in controlling the human immune system and inflammatory cascade is the TLR signaling pathway. As a defence mechanism, TLRs activation triggers signaling pathways; repairs injured tissue, and frequently release various inflammatory cytokines and immunological modulators. TLRs in and around wounds regulate inflammatory generation and the function of the innate immune system. Studies have indicated that elevated TLRs 2 and 4 expression in macrophages obtained from the bone marrow of non-obese diabetic rats is linked to proinflammatory cytokines and NF-κB activation [39]. The metabolism of glucose, cell differentiation, proliferation, and inflammatory responses are all linked to the NFKB signaling pathway. By modifying various NFKB pathways, the expression of cytokines and inflammatory chemicals contributes significantly to the pathogenesis of diabetes and its related microvascular and macrovascular consequences. By promoting the synthesis of proinflammatory factors, NFKB takes part in the inflammatory phase of the wound healing process. Therefore, controlling variables associated with the NFKB signaling pathway can control the inflammatory response during wound healing. Patients with diabetic foot ulcers can effectively increase their wound healing efficiency with this method [40].

The VEGF signaling pathway is a protein that stimulates vasculogenesis and angiogenesis. Decreased levels of VEGF receptor-2 are thought to cause poor wound healing [41]. Therefore, VEGF has been used as a biomarker of DW healing. VEGF stimulates several cellular signaling pathways, including the Akt 1 pathway, for various cellular functions that accelerate wound healing. An interaction value of 0.957 suggests that quercetin may transport chemical signals that the VEGF receptor protein receives and uses to activate the Akt-1 signaling pathway. Akt-1 is a vital component of the Pi3K/Akt signaling pathway and regulates many stresses, including metabolism, proliferation, migration, growth, and angiogenesis [42].

A class of serine/threonine protein kinases involved in the immunological response, inflammation, cell proliferation, apoptosis, and hormone signaling is known as the MAPK signaling pathway. It has been established that there are three main subfamilies of MAPK genes: p38 MAPKs, JNK, and Extracellular Signal-Regulated Kinases (ERK). ERK, JNK, and p38 MAPK are the three primary subfamilies of MAPK genes. According to theories, autophagy is inactivated with a downregulation of the p38/MAPK pathway, preventing keratinocyte migration in conditions with elevated glucose levels. This demonstrates the critical function of the MAPK pathway in the healing of DW [35].

TGF-β1 and TGF-β2 are key members of the TGF-β superfamily crucial in wound healing. TGF-β is present in wound fluid in several investigations. TGF-β performs various regulatory cellular tasks at the cellular regulatory level, including drawing fibroblasts and macrophages to the wound site to aid in healing. Furthermore, TGF-β plays a crucial role in wound healing in DW, although it is not significantly elevated compared to other reduced growth factors, which could postpone healing [37].

In silico approaches have various advantages, including lowering the number of compounds or molecules, accelerating the discovery of most compound-protein interactions via database searches, and reducing the need for laboratory animals in predictive studies. In silico prediction not only saves time in developing ligand-based medications and serves as a starting point for *in vitro* and *in vivo* testing but also supports *N. sativa* L. and *T. foenum-graecum* as therapeutic candidates, particularly for DW. However, the limitations of this method must be recognized, such as the enormous volume of data processed. High accuracy is essential, and the danger of bias varies according to the database utilized for predictions. Some possible active metabolites of *N. sativa* L. and *T. foenum-graecum* that are not currently included in the database may raise the risk of prediction mistakes and diminish the accuracy of study results.

CONCLUSION

Network pharmacology analysis of *N. sativa* L. and *T. foenum-graecum* revealed a correlation between their metabolites, surface targets or receptors, intracellular proteins, and DW signaling pathways. This suggests that both plants have potential as drug candidates for DW. This study can be further developed with potential clinical application for DW healing.

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AUTHORS CONTRIBUTIONS

The authors acknowledge their contributions to this research as follows: Research conception and design: RS, RA; Data collection: MRD, SQA Analysis and interpretation of results: MRD, SQA; Preparation of manuscript draft: MRD, SQA. All authors reviewed the results and approved the final version of the manuscript.

CONFLICTS OF INTERESTS

No potential conflict of interest relevant to this article was reported.

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