Network Pharmacology-Based Prediction of the Mechanisms of a Traditional Thai Herbal Formula for Diabetes Treatment

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Abstract

Diabetes is among the top five leading causes of mortality in Thailand, with a rapidly increasing number of affected individuals each year. Traditional Thai medicine has long utilized a polyherbal formulation composed of honey, *Andrographis paniculata*, *Tinospora crispa* (L.), *Curcuma longa* L., and *Orthosiphon aristatus* for diabetes management. These medicinal herbs are known for their hypoglycemic and antioxidant properties. However, the pharmacological mechanisms, biological activities, and phytochemical profiles of this formulation remain largely unexplored. This study employs network pharmacology to predict the potential biological activities of a 40% ethanol extract of the herbal formula. Swiss TargetPrediction was utilized to identify target proteins, while KEGG pathway enrichment analysis was conducted to map relevant signaling pathways. Additionally, protein-protein interaction (PPI) networks were constructed using the STITCH database to elucidate molecular interactions. The findings suggest that the herbal formula exerts its therapeutic effects through multiple pathways, including nitrogen metabolism, steroid hormone biosynthesis, ovarian steroidogenesis, EGFR tyrosine kinase inhibitor resistance, cancer-related pathways, endocrine resistance, and metabolic pathways. These insights provide a scientific foundation for further experimental validation and the potential integration of this traditional formulation into diabetes management strategies.

Keywords: Traditional Thai medicine / Diabetes treatment / Herbal formula / Molecular mechanisms / Pathway enrichment analysis

1. Introduction

Diabetes is among the top five most lethal diseases, resulting in the highest number of mortality rate among Thais. In 2024, a total of 14,341 individuals people died to the disease [1]. Diabetes has emerged as a significant public health concern in Asia and globally. At present, around 537 million individuals globally are living with diabetes, and the number is increasing annually [2].

Synthetic antidiabetic medications, despite generally used, frequently exhibit numerous limitations, including unpleasant effects, elevated treatment costs, and a restricted ability for dealing with the complicated, multifactorial aspects of diabetes, such as oxidative stress, chronic inflammation, and insulin resistance. These limitations have encouraged an increasing interest in traditional medicine as a supplemental or alternative treatment. In traditional Thai medicine, multi-herbal preparations have long been utilized to control diabetes and related metabolic diseases. One such formulation includes honey, *Andrographis paniculata*, *Tinospora crispa* (L.), *Curcuma longa* L., and *Orthosiphon aristatus*. Each component is known for its individual antidiabetic effects. Honey contains antioxidants that reduce oxidative stress [3] and exhibits α-amylase and α-glucosidase inhibitory activities that help lower blood sugar levels [4]. A. *paniculata* has demonstrated antioxidant effects [5] and inhibition of carbohydrate-digesting enzymes, contributing to blood glucose reduction [6]. T. *crispa* exhibits antioxidant properties [7], reduces blood glucose levels in diabetic rats, and stimulates pancreatic β-cell regeneration [8]. C. *longa* contains curcumin, known for its antioxidant and anti-inflammatory [9]. It also enhances β-cell function and promotes insulin sensitivity, which is essential for the management of type 2 diabetes [8]. O. *aristatus* demonstrates inhibitory effects on α-amylase and α-glucosidase, essential enzymes in carbohydrate digestion, consequently reducing glucose absorption [10]. Although this multi-herb mixture has been utilized in Thai traditional medicine for a long time, most of current research has focused on the pharmacological effects of its individual ingredients in isolation. A significant research gap remains in understanding the synergistic mechanisms and therapeutic potential of the whole mixture. Therefore, a systems-level analysis using network pharmacology is essential to elucidate the molecular mechanisms and multi-target interactions underlying this traditional Thai herbal formula's antidiabetic efficacy.

Network Pharmacology is a methodology employed to investigate and understand the effects of compounds or drugs within biological systems by analyzing the network of interacting targets. Network Pharmacology facilitates the prediction of synergistic effects when numerous drugs are concurrently delivered [11]. This method provides insights into the intricate interactions between active compounds in medicinal plants, enabling the design of herbal remedies with enhanced therapeutic efficacy [12]. Network pharmacology is essential for enhancing treatment outcomes and progressing herbal medicine and drug development by delineating the interactions among bioactive chemicals inside biological networks [11].

2. Objective

1. Applies network pharmacology to predict biological activities of Thai herbal diabetes formula.
2. Identifies interactions between compounds, targets, and pathways.
3. Aims to scientifically support traditional herbal medicine for future antidiabetic therapies.

3. Scope of study

This study aims to explore the antidiabetic mechanisms of a traditional Thai herbal formula using a network pharmacology approach. The scope includes identifying bioactive compounds, predicting diabetes-related targets, constructing interaction networks, and analyzing relevant pathways to reveal multi-target therapeutic actions.

4. Materials and Methods

4.1 Data collection and preparation

The bioactive constituents of antidiabetic formulations containing honey, T. *crispa*, A. *paniculata*, C. *longa*, and O. *aristatus* were identified through pharmacological characterization studies based on literature and database searches, in order to analyze the compounds and predict their biological pathways.

4.2 Target identification

The SwissTargetPrediction online tool was used to identify the target once the canonical SMILES of bioactive chemicals were obtained. Targets were chosen if their likelihood score was higher than 0.1.

4.3 Gene Ontology and Pathway Enrichment Analysis

ShinyGO 0.81, an online platform for enrichment analysis across different species, was used to conduct Gene Ontology (GO) and route enrichment investigations. Finding biological processes, molecular roles, and cellular constituents associated with the targets of the compounds in the extract was the goal of the analysis. KEGG pathways and GO keywords were considered significantly enriched if their false discovery rate (FDR) was less than 0.05.

4.4 Protein-Protein Interaction (PPI) Network Construction

To forecast protein-protein interactions between the targets of the bioactive chemicals in the extract, the STITCH database was used.

5. Result and discussion

5.1 GO and KEGG enrichment analysis

KEGG pathway enrichment analysis was conducted using ShinyGO 0.81 to elucidate the biological activities and possible processes of the target proteins. Figure 1A illustrates the most enriched routes. The most notably enriched pathways (FDR-adjusted $p < 0.05$) encompassed nitrogen metabolism, steroid hormone biosynthesis, ovarian steroidogenesis, resistance to EGFR tyrosine kinase inhibitors, progesterone-mediated oocyte maturation, and several cancer-related pathways, including prostate cancer and microRNAs in cancer. Significantly, neuroactive ligand-receptor interactions, phospholipase D signaling, and the PI3K-Akt signaling pathway demonstrated substantial enrichment, suggesting their potential role in metabolic and signaling processes pertinent to diabetes. The bubble plot demonstrates that these pathways are considerably enriched (as indicated by -log10 (FDR)) and encompass a substantial number of genes, with as many as 50 genes associated with specific pathways. Fold enrichment data further substantiate the robust correlations between the chemical targets and various biological pathways. Hierarchical clustering of the enriched pathways (Figure 1A) categorized them into many clusters according to functional similarity. Cancer-related, hormone-related, and signaling pathways, including PI3K-Akt, cAMP, and calcium signaling, were

strongly associated, indicating integrated biological processes. This clustering indicates a multi-target therapeutic potential of the herbal combination, specifically in regulating insulin resistance, inflammation, and oxidative stress-key features of diabetes pathogenesis.

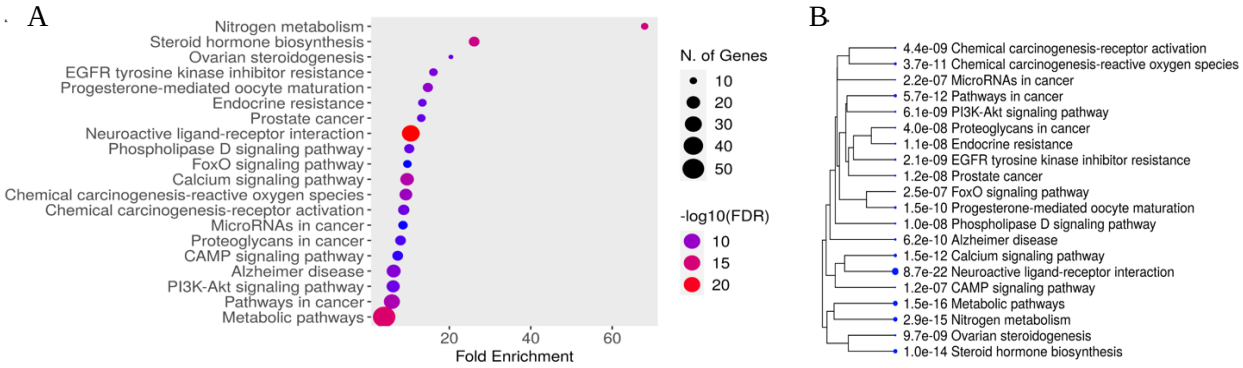

Figure 1. Shows (A) Biological pathways associated with biological compounds and (B) Relationship between biological pathways associated with biological compounds.

5.2 Pathway Enrichment Analysis of Targets Related to Type 2 Diabetes

The analysis of herbal extracts for treating diabetes revealed several biological pathways associated with type 2 diabetes, particularly those involved in metabolism and hormone signaling processes. The nitrogen metabolism pathway, which exhibited the highest fold enrichment value, suggests that the studied gene sets are strongly associated with nitrogen metabolism an essential process for maintaining cellular energy balance. The steroid hormone biosynthesis and ovarian steroidogenesis pathways showed fold enrichment values of 26.1 and 20.4, respectively. Both are involved in the synthesis of steroid hormones such as cortisol, which plays a crucial role in regulating blood glucose levels. Additionally, several signaling pathways implicated in type 2 diabetes were identified, including the phospholipase D signaling pathway, calcium signaling pathway, and PI3K-Akt signaling pathway, all of which are key regulators of insulin activity and insulin resistance. The FoxO signaling pathway was also enriched and is known to play a role in glucose metabolism and oxidative stress resistance. Moreover, cancer-related pathways were observed, such as prostate cancer, proteoglycans in cancer, and chemical carcinogenesis, indicating a potential overlap in signaling mechanisms between metabolic disorders and cancer development.

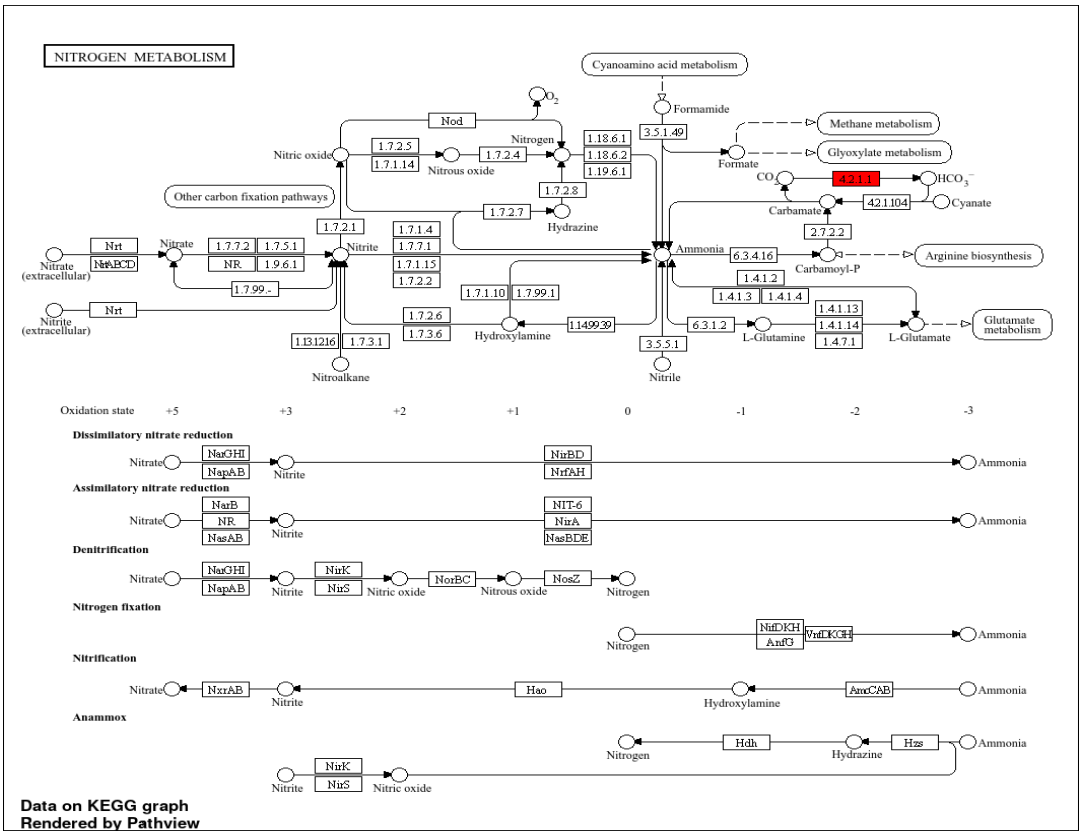

Figure 2. hows the pathway schematic of antioxidant targets from the Thai herbal formula for diabetes from the nitrogen metabolism pathway.

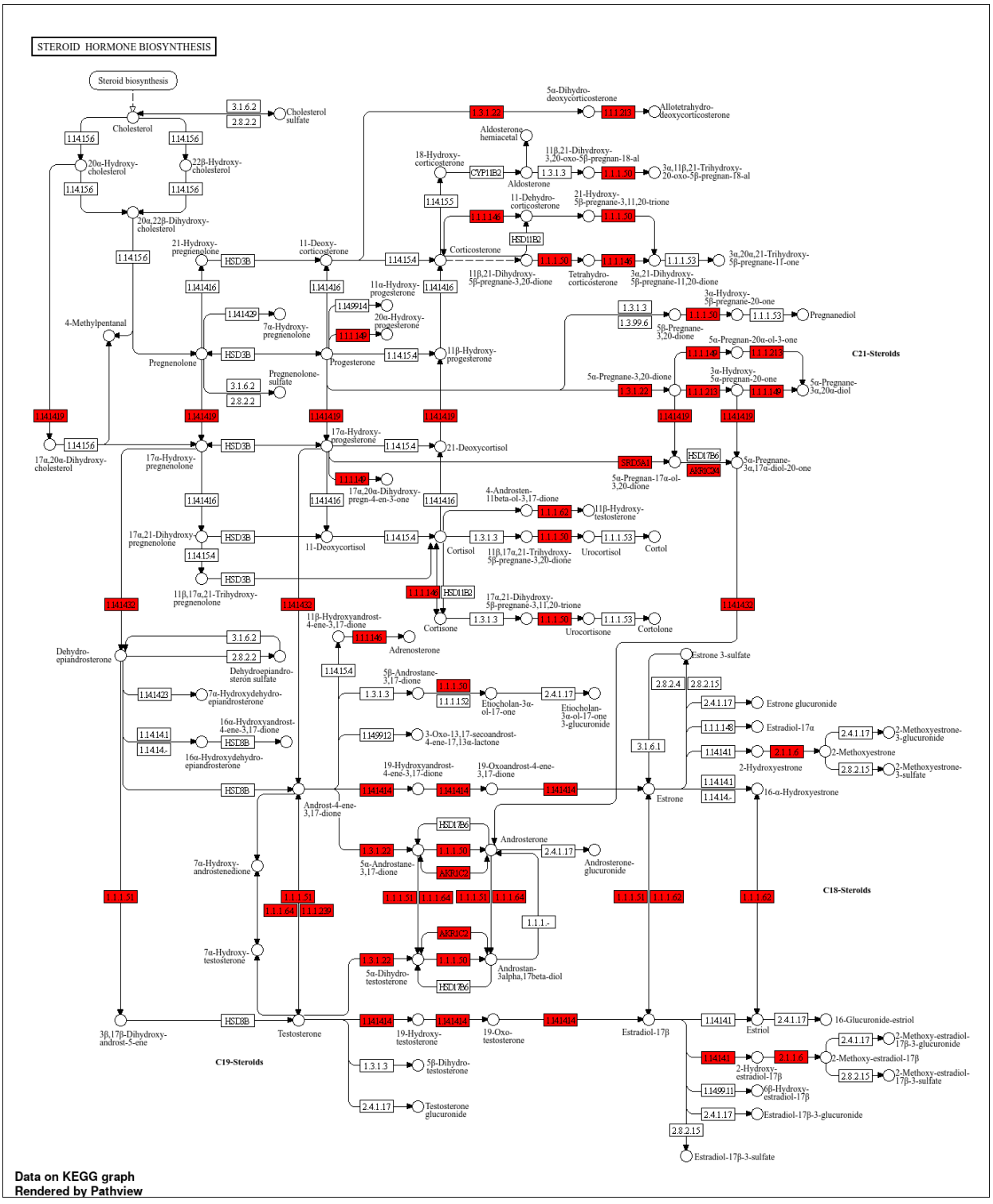

Figure 3. hows the pathway schematic of antioxidant targets from the Thai herbal formula for diabetes from the Steroid hormone pathway.

5.3 The protein-protein interaction (PPI) network analysis

The predicted targets of the antidiabetic herbal formula displayed a highly linked network, suggesting general interactions among associated proteins. In the network, nodes represent proteins, and pill-shaped nodes indicate known drug associations. Edges (connecting lines) demonstrate the interactions between proteins. Several key target proteins identified within the network are implicated in inflammation and apoptosis, including CST3, SERPINA6, and MCL1. Key signaling proteins, including AKT1, mTOR, MAPK1, and EGFR, were discovered as pivotal nodes within the PI3K-Akt, FOXO, and MAPK signaling pathways, which are directly involved in the regulation of glucose production, cellular growth, and insulin resistance. The solid interconnectivity within the network demonstrates the potential of the herbal formula to exert effects through multiple mechanisms and pathways, highlighting the pharmacological complexity of multi-component herbal remedies. This systems-level analysis suggests that the formula may achieve therapeutic efficacy by simultaneously modulating a network of interacting proteins, resulting in synergistic biological effects.

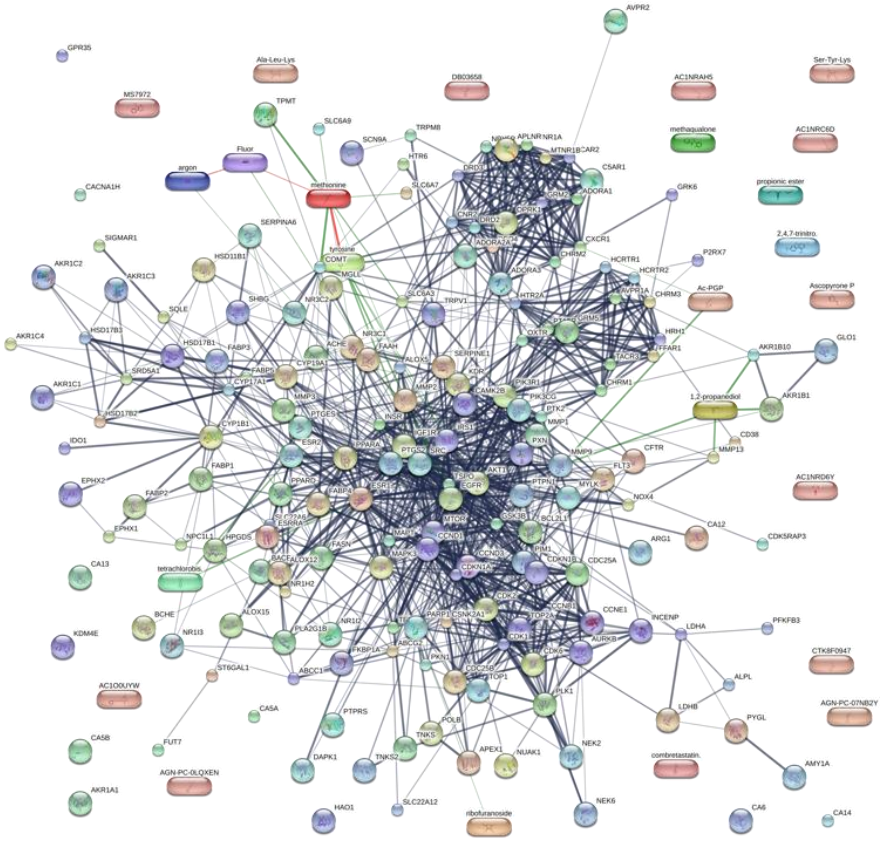

Figure 4. Protein-protein interaction (PPI) network analysis of the predicted targets of Predicted Targets from the Antidiabetic Herbal Formula.

5.4 Discussion

Pathway enrichment study of herbal extracts demonstrated high enrichment of genes related with type 2 diabetes, counting those implicated in metabolic processes, cell signaling, and oxidative stress response mechanisms, all of which are essential in the pathogenesis of the disease. The nitrogen metabolism pathway affected by the herbal extracts is linked to the activity of Carbonic Anhydrase (EC 4.2.1.1), an enzyme that catalyzes the conversion of carbon dioxide (CO_2) into bicarbonate (HCO_3^-). Bicarbonate is essential in gluconeogenesis, the metabolic process of synthesizing new glucose in the liver [13]. A study established that modulating Carbonic Anhydrase activity in diabetic mice led to decrease blood glucose levels [14].

The herbal formula may affect the enzyme 11β-HSD1 (EC 1.1.1.146) in the Steroid Hormone Biosynthesis pathway, which is associated with heightened insulin resistance and an elevated risk of type 2 diabetes when activated [15]. This pathway is also involved in the synthesis of estradiol via the enzyme CYP19A1 (Aromatase), which converts androgens, such as testosterone into estrogens, including estradiol. Estradiol is crucial for the regulation of glucose metabolism and improvement of insulin sensitivity [16]. Another relevant pathway is Ovarian Steroidogenesis, which regulates steroid synthesis in the ovaries. The herbal formula may affect key regulatory proteins, including INSR (Insulin Receptor), which mediates insulin binding and enhances insulin sensitivity, consequently contributing in the regulation of blood glucose levels [17].

The Calcium Signaling Pathway is essential for regulating insulin secretion from pancreatic β-cells, particularly through CaV1–CaV3 calcium channels, which act as key signals for insulin exocytosis [18]. The herbal formula may affect proteins including RTKs and PI3K in the Phospholipase D (PLD) Signaling Pathway, which are crucial for insulin signaling and glucose uptake [19].

Finally, the herbal formula may influence insulin signaling processes within the PI3K-Akt Signaling Pathway, enhancing glucose uptake into cells. Concurrently, the FOXO Signaling Pathway plays a role in regulating gene expression involved in oxidative stress response, gluconeogenesis, and apoptosis of pancreatic β-cells [20].

Protein–protein interaction (PPI) network analysis revealed a highly interconnected framework, indicating close relationships among multiple proteins involved in key biological processes. The results suggest that the herbal formula may exert its effects by simultaneously stimulating or inhibiting multiple signaling pathways. The network emphasized interactions among proteins associated with oxidative stress response, FOXO, PI3K-Akt, MAPK signaling, and apoptosis regulatory pathways, which are crucial in the pathogenesis of type 2 diabetic mellitus (T2DM).

These findings support the hypothesis that the herbal formula acts via a multi-target, multi-pathway approach, rather than through modulation of a single molecular target. This mode of action may enhance therapeutic efficacy while potentially reducing adverse effects commonly associated with single-target agents.

However, it is important to note that the molecular interactions identified through network analysis remain predictive and exploratory in nature. To address these limitations, future research should integrate experimental validation, such as in vitro assays, in vivo models, and clinical investigations, to confirm predicted mechanisms and targets. Incorporating metabolomics, proteomics, and molecular docking studies

can further enhance the mechanistic understanding and provide complementary evidence. Moreover, the development of more comprehensive and curated databases specific to traditional herbal medicine will improve the reliability of network pharmacology predictions. Ultimately, a multi-disciplinary approach combining computational and experimental techniques is essential to fully elucidate the therapeutic potential of traditional herbal formulations.

6. Conclusion

The results of the analysis suggest that the Antidiabetic herbal formula has the potential to exert its antidiabetic effects through multiple molecular targets and diverse biological pathways implicated in type 2 diabetes mellitus (T2DM). In particular, the FOXO, PI3K-Akt, and calcium signaling pathways key regulators of glucose homeostasis and insulin sensitivity were notably enriched. However, further experimental investigations, including cellular, animal, and clinical studies, are required to validate these predicted mechanisms and to establish the therapeutic efficacy and safety of the formula in a systematic and evidence-based manner.

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