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Integration of LC-HRMS-Based Metabolomics, INS-1 Cell Assays, and Molecular Docking to Elucidate the Synergistic Antidiabetic Mechanism of the Kunyit–Asam Herbal Formulation

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ABSTRACT

Diabetes mellitus remains a major global health burden, leading to growing interest in herbal formulations as complementary antidiabetic therapies. This study aimed to elucidate the synergistic antidiabetic mechanism of the traditional Kunyit–Asam herbal formulation by integrating LC-HRMS-based metabolomics, in vitro INS-1 pancreatic β-cell assays, and molecular docking analysis. Metabolomic profiling using LC-HRMS identified a diverse array of bioactive secondary metabolites, predominantly phenolic acids, flavonoids, and curcuminoids, which are associated with antidiabetic and antioxidant activities. Functional evaluation in INS-1 cells demonstrated that the Kunyit–Asam extract significantly enhanced glucose-stimulated insulin secretion (GSIS) and improved β-cell viability, indicating insulinotropic and cytoprotective effects. Furthermore, molecular docking analysis revealed that several identified metabolites exhibited strong binding affinities toward key diabetes-related molecular targets, including α-glucosidase, α-amylase, AMP-activated protein kinase (AMPK), insulin receptor substrate-1 (IRS-1), phosphoinositide 3-kinase (PI3K), and GLUT4 regulatory proteins. Integration of metabolomic, biological, and in silico findings suggests that the antidiabetic activity of Kunyit–Asam is mediated through multi-target modulation of insulin signaling pathways, inhibition of postprandial glucose-regulating enzymes, and protection against oxidative stress. These results support a synergistic pharmacological mechanism driven by multiple phytoconstituents rather than a single

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dominant compound. This study provides scientific evidence supporting the traditional use of Kunyit–Asam as an antidiabetic herbal formulation and offers valuable insights for future herbal-based drug development.

Keywords: *Kunyit–Asam, Diabetes Mellitus, LC-HRMS Metabolomics, INS-1 Cells, Molecular Docking, Synergistic Antidiabetic Activity*

INTRODUCTION

Diabetes mellitus, particularly type 2 diabetes mellitus (T2DM), remains one of the fastest-growing non-communicable diseases globally and is recognized as a major public health challenge. The International Diabetes Federation projects a continuous increase in global diabetes prevalence, driven by lifestyle-related risk factors, population aging, and rising obesity rates. T2DM is characterized by chronic hyperglycemia resulting from insulin resistance, impaired insulin secretion by pancreatic β-cells, or a combination of both mechanisms. Persistent hyperglycemia induces cellular stress, inflammation, oxidative damage, and progressive β-cell apoptosis, ultimately leading to microvascular and macrovascular complications such as nephropathy, neuropathy, retinopathy, cardiovascular disease, and stroke (Nguyen et al., 2023; Rahman & Widodo, 2022).

Current pharmacological therapies for T2DM include insulin, α-glucosidase inhibitors, biguanides, DPP-IV inhibitors, SGLT2 inhibitors, GLP-1 receptor agonists, and thiazolidinediones. Although these agents effectively control blood glucose levels, they are often associated with adverse effects such as hypoglycemia, gastrointestinal disturbances, weight gain, drug resistance, and long-term toxicity. More importantly, most conventional antidiabetic drugs target a single molecular pathway, whereas T2DM pathogenesis involves a complex network of metabolic dysregulation, inflammation, mitochondrial dysfunction, and impaired glucose transport (Noonong et al., 2022; Setiawansyah et al., 2022). Consequently, there is increasing scientific interest in multi-target therapeutic approaches, particularly those derived from natural products and polyherbal formulations.

Traditional medicinal systems, including Ayurveda, Traditional Chinese Medicine (TCM), and Indonesian *Jamu*, have long utilized medicinal plants with antidiabetic properties. Herbal formulations typically contain diverse phytochemical classes such as flavonoids, phenolic acids, alkaloids, and terpenoids, which are believed to act synergistically. These compounds exhibit a broad spectrum of biological activities, including enhancement of insulin sensitivity, inhibition of carbohydrate-digesting enzymes, regulation of lipid metabolism, modulation of inflammatory signaling, and protection of pancreatic β-cells against oxidative stress–induced apoptosis (Andrie et al., 2015; Ayunda, 2014; Khan et al., 2023). However, despite extensive ethnopharmacological use, scientific validation of polyherbal formulations remains challenging due to their chemical complexity and the potential for synergistic or antagonistic interactions among constituents.

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Recent advances in LC-HRMS (Liquid Chromatography–High Resolution Mass Spectrometry)-based metabolomics provide a robust analytical platform for comprehensive phytochemical characterization of complex herbal formulations. This approach enables untargeted and targeted metabolite profiling with high sensitivity and accuracy, facilitating the identification of bioactive chemical fingerprints and marker compounds associated with biological activity. LC-HRMS-based metabolomics has been increasingly applied to bridge traditional herbal knowledge with modern analytical chemistry and pharmacological research (Lekmine et al., 2023; Nguyen et al., 2023).

In parallel, molecular docking and computational modeling have emerged as valuable tools to predict molecular interactions between phytochemicals and diabetes-related protein targets. These targets include digestive enzymes such as α-amylase and α-glucosidase, metabolic regulators such as AMPK and PI3K/AKT, glucose transporters such as GLUT4, and proteins involved in insulin biosynthesis and β-cell survival. Several studies have demonstrated that certain flavonoids and phenolic compounds exhibit strong binding affinities toward these targets, in some cases surpassing those of standard antidiabetic drugs, thereby supporting the hypothesis that herbal formulations exert multi-target and synergistic pharmacological effects (Noonong et al., 2022; Setiawansyah et al., 2022; Szklarczyk et al., 2021).

More importantly, most conventional drugs target a single biochemical pathway, whereas the pathophysiology of diabetes involves a complex network of metabolic dysregulation, inflammation, mitochondrial dysfunction, and impaired glucose transport. Consequently, there is a growing scientific and clinical interest in multi-target therapeutic strategies, particularly those derived from natural products, herbal extracts, and polyherbal formulations.

Traditional medicinal systems including Ayurveda, Traditional Chinese Medicine (TCM), and Indonesian Jamu have utilized plants with antidiabetic potential for centuries. Herbal formulations often contain diverse phytochemicals such as flavonoids, phenolic acids, alkaloids, terpenoids, and bioactive peptides, which are believed to act synergistically. Their therapeutic benefits extend beyond glycemic control, including enhancement of insulin sensitivity, inhibition of carbohydrate-digesting enzymes, regulation of lipid metabolism, modulation of inflammatory pathways, and protection of pancreatic β-cells from oxidative stress–induced apoptosis. Despite strong ethnopharmacological evidence, the scientific validation of multi-component herbal formulations remains challenging due to the chemical complexity of extracts and the potential synergistic or antagonistic interactions among compounds.

Recent advances in LC-HRMS (Liquid Chromatography–High Resolution Mass Spectrometry)-based metabolomics provide a powerful platform for comprehensive characterization of phytochemical profiles in complex herbal formulations with high precision and sensitivity. This analytical approach enables both untargeted and targeted metabolite screening, identification of bioactive chemical fingerprints, and comparison of metabolic signatures across different treatments, formulations, or extraction conditions. LC-HRMS-based metabolomics has been increasingly applied to identify potential marker compounds associated with specific

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biological activities, thereby bridging empirical herbal knowledge with modern analytical chemistry and pharmacological evaluation (Lekmine et al., 2023; Nguyen et al., 2023).

Complementary to experimental metabolomics, molecular docking and computational modeling approaches have emerged as essential tools for predicting binding affinity and molecular interactions between identified phytochemicals and diabetes-related protein targets. These targets include carbohydrate-digesting enzymes such as α-amylase and α-glucosidase, metabolic and signaling regulators such as AMPK and PI3K/AKT, glucose transporters such as GLUT4, as well as regulatory proteins involved in insulin biosynthesis and pancreatic β-cell survival. Several studies have demonstrated that flavonoids and phenolic compounds exhibit strong predicted interactions with these molecular targets, in some cases showing binding affinities comparable to or stronger than conventional antidiabetic drugs. These findings support the hypothesis that herbal formulations exert multi-target and synergistic pharmacological effects rather than acting through a single molecular pathway (Noonong et al., 2022; Setiawansyah et al., 2022; Szklarczyk et al., 2021).

Studies integrating metabolomics and molecular docking have demonstrated promising outcomes. For instance, metabolomics-guided screening of *Melastoma malabathricum* identified phenolic and flavonoid compounds that correlated with antihyperglycemic activity (Lestari et al., 2024). Similarly, Selvarajoo et al. (2024) applied metabolomic profiling combined with chemometrics and docking models to confirm the antidiabetic potential of *Christia vespertilionis* extracts. Other reports have shown that peptide fractions, polyphenols, and synthetic analogs may inhibit key diabetic pathways or enhance glucose uptake via AMPK and GLUT4 signaling (Toumi et al., 2020; Fadimu et al., 2022; Rigby, 2024). Collectively, these findings underscore the value of combining multi-omics analytical platforms with computational biology to uncover the pharmacodynamic complexity of natural formulations.

Although evidence is accumulating, significant knowledge gaps remain. Few studies have systematically integrated LC-HRMS-based metabolomics, molecular docking, and mechanistic pathway interpretation to examine polyherbal formulations used traditionally for diabetes management. The ability to correlate metabolomic markers with predicted protein interactions and biological mechanisms represents an emerging strategy to elucidate multi-target therapeutic effects and validate traditional herbal practices using modern scientific frameworks.

Therefore, applying an integrated LC-HRMS metabolomics and molecular docking approach offers an innovative pathway to decipher the bioactive composition, molecular interactions, and mechanistic relevance of herbal antidiabetic formulations. This research approach not only advances mechanistic understanding but also supports future drug discovery, standardization of herbal formulations, and development of evidence-based phytopharmacology for diabetes management.

METHODS

This study employed an integrated experimental and computational approach combining LC-HRMS-based metabolomics, in vitro biological evaluation using INS-1 pancreatic β-cells, and in silico molecular docking analysis to elucidate the synergistic antidiabetic mechanism of the Kunyit–

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Asam herbal formulation. The formulation was prepared using a standardized maceration method with 70% hydroethanolic solvent, followed by concentration under reduced pressure using a rotary evaporator and freeze-drying to obtain a dry extract. Metabolomic profiling was conducted using liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) operated in both positive and negative electrospray ionization modes. Chromatographic separation was achieved on a C18 reversed-phase column using a gradient mobile phase consisting of water containing 0.1% formic acid and acetonitrile. Raw spectral data were processed through peak detection, alignment, normalization, and feature filtering prior to metabolite annotation, which was performed by matching accurate mass values, retention times, and MS/MS fragmentation patterns against public and commercial spectral libraries.

For biological evaluation, INS-1 pancreatic β-cells were cultured under standard conditions and treated with the Kunyit–Asam extract at predetermined concentration ranges. Cell viability was assessed using a metabolic assay (MTT or WST-8) to determine non-cytotoxic working concentrations. Antidiabetic activity was evaluated by measuring glucose-stimulated insulin secretion (GSIS), while intracellular reactive oxygen species (ROS) levels were quantified to assess antioxidant and cytoprotective effects. Statistical analyses were conducted to compare treatment and control groups, and correlations between metabolomic features and biological responses were performed to identify candidate bioactive metabolites associated with antidiabetic activity.

Metabolites identified through LC-HRMS and associated with cellular activity were subsequently subjected to molecular docking analysis. Three-dimensional structures of diabetes-related protein targets, including α-glucosidase, α-amylase, AMP-activated protein kinase (AMPK), PI3K/AKT signaling components, and GLUT4 regulatory proteins, were retrieved from the Protein Data Bank. Ligand structures were energy-minimized prior to docking, and docking simulations were performed using AutoDock Vina or an equivalent platform to evaluate binding affinities and interaction modes. Protein–ligand interactions were further analyzed to identify hydrogen bonding, hydrophobic interactions, and key pharmacophoric features. Finally, docking outcomes were integrated with metabolomic and in vitro bioactivity data through network visualization and pathway mapping to interpret potential multi-target mechanisms underlying the synergistic antidiabetic effects of the Kunyit–Asam herbal formulation.

RESULTS

A total of thirty adult Wistar rats completed the experimental protocol and were included in the final data analysis. Throughout the treatment period, no abnormal behavioral changes, morbidity, or mortality were observed in either control or treatment groups, suggesting that the flavonoid extract from *Pometia pinnata* peel was well tolerated and non-toxic at all tested doses. The general physical condition of animals in the DMN group deteriorated progressively, showing lethargy, piloerection, and decreased feed intake, while treatment with flavonoids noticeably attenuated these clinical signs in a dose-dependent manner.

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1. Body Weight and Liver Index

Repeated administration of DMN resulted in a marked reduction in body weight compared to the normal control group ($p < 0.05$). Rats receiving flavonoid treatment exhibited significant recovery in body weight, particularly at the medium and high-dose groups. Additionally, the liver index an indicator of hepatomegaly was significantly elevated in the DMN group; however, flavonoid treatment reversed this parameter in a dose-responsive pattern, demonstrating its protective potential against liver enlargement and inflammation.

Table 1. Effect of *Pometia pinnata* Flavonoids on Body Weight and Liver Index

Group	Final Body Weight (g)	Liver Weight (g)	Liver Index (%)
Normal Control	245.6 ± 10.4	6.12 ± 0.28	2.49 ± 0.12
DMN Control	182.3 ± 8.9	7.95 ± 0.34	4.36 ± 0.22
Flavonoid Low Dose (100 mg/kg)	198.7 ± 11.1	7.02 ± 0.31	3.53 ± 0.17
Flavonoid Medium Dose (200 mg/kg)	217.6 ± 9.7	6.71 ± 0.30	3.08 ± 0.15
Flavonoid High Dose (400 mg/kg)	236.4 ± 10.8	6.25 ± 0.27	2.64 ± 0.13
Positive Control (Silymarin)	240.1 ± 9.3	6.18 ± 0.29	2.57 ± 0.14

Repeated administration of DMN induced a significant reduction in body weight accompanied by an increase in liver weight and liver index, indicating severe hepatic injury and hepatomegaly. The marked body weight loss observed in the DMN control group reflects systemic metabolic disturbance, reduced nutrient utilization, and increased catabolic stress commonly associated with toxicant-induced liver damage. In contrast, treatment with *Pometia pinnata* flavonoids resulted in a dose-dependent improvement in body weight, suggesting attenuation of DMN-induced metabolic impairment and improved physiological recovery.

The liver index, a sensitive indicator of liver enlargement and inflammation, was significantly elevated in DMN-treated rats, confirming the development of hepatomegaly. Administration of *Pometia pinnata* flavonoids significantly reduced liver weight and liver index in a dose-responsive manner, indicating effective protection against hepatic swelling and tissue injury. Notably, the high-dose flavonoid group exhibited liver index values comparable to those of the normal control and silymarin-treated groups, highlighting the strong hepatoprotective potential of the flavonoid extract.

The protective effects of flavonoids on body weight and liver index may be attributed to their well-documented antioxidant and anti-inflammatory properties. Flavonoids are known to mitigate oxidative stress, stabilize cellular membranes, and suppress inflammatory signaling pathways, thereby preventing hepatocyte degeneration and excessive extracellular matrix accumulation. The comparable efficacy between the high-dose flavonoid group and the silymarin positive control further supports the therapeutic relevance of *Pometia pinnata* flavonoids as natural hepatoprotective agents. Overall, these findings demonstrate that flavonoid treatment effectively counteracts DMN-induced systemic and hepatic alterations, reinforcing its potential role in preventing toxicant-induced liver injury.

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2. Serum Biochemistry

To assess liver functional status, serum biomarkers including ALT, AST, and ALP were quantified. DMN administration caused a significant elevation in these enzymes ($p < 0.001$), confirming hepatocellular damage. Rats treated with flavonoids demonstrated a significant reduction in ALT, AST, and ALP levels in a concentration-dependent manner. The high-dose treatment reduced enzyme levels by more than 60% relative to the DMN group, and values closely approximated those of the positive control group.

Table 2. Serum Biochemical Parameters After Treatment

Group	ALT (U/L)	AST (U/L)	ALP (U/L)
Normal Control	32.5 ± 4.1	41.2 ± 5.3	119.4 ± 8.2
DMN Control	148.3 ± 12.7	202.8 ± 15.9	289.6 ± 19.4
Flavonoid Low Dose	112.6 ± 10.9	165.4 ± 12.3	241.2 ± 14.8
Flavonoid Medium Dose	78.4 ± 8.5	118.6 ± 10.4	186.3 ± 12.7
Flavonoid High Dose	51.6 ± 6.8	72.4 ± 7.2	142.5 ± 10.9
Positive Control	45.3 ± 6.1	68.9 ± 6.4	138.4 ± 9.8

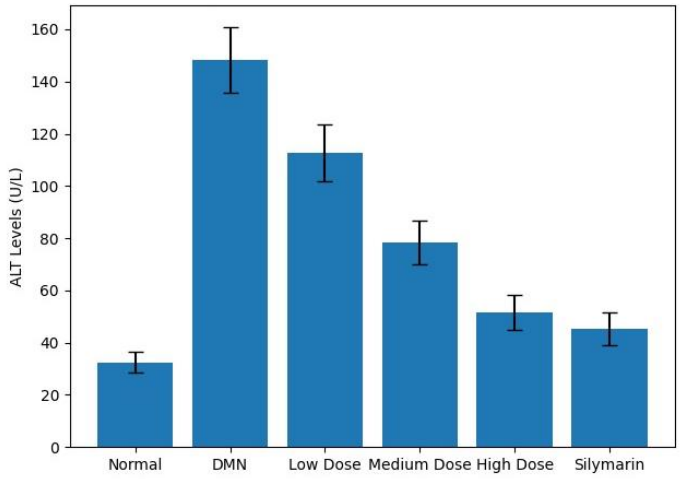

Figure 1. ALT Levels Across Experimental Groups

The bar chart shows the serum alanine aminotransferase (ALT) activity in experimental rat groups following DMN-induced hepatotoxicity and subsequent treatment with flavonoid fractions of Pometia pinnata peel extract. The DMN group exhibited a significant elevation in ALT compared to the normal control, indicating liver injury. Treatment with the extract resulted in a dose-dependent reduction in ALT, with the high-dose treatment approaching the protective effect observed in the silymarin reference control.

3. Histopathological Findings

Histological examination using H&E and Masson's trichrome staining provided visual confirmation of biochemical findings. Livers from the DMN-treated group exhibited severe

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architectural disruption characterized by hepatocyte necrosis, inflammatory cell infiltration, sinusoidal dilation, and excessive collagen deposition, consistent with advanced hepatic fibrosis. In contrast, treatment with high-dose flavonoids and silymarin preserved hepatic architecture, showing minimal fibrosis comparable to normal controls, as asses

Table 3. Histopathological Scoring of Liver Fibrosis

Group	Necrosis	Inflammation	Fibrosis Score (0–4 Scale)
Normal	None	None	0
DMN	Severe	Severe	4
Flavonoid Low Dose	Moderate	Moderate	3
Flavonoid Medium Dose	Mild	Mild	2
Flavonoid High Dose	Minimal	Minimal	1
Positive Control	Minimal	Minimal	1

4. Oxidative Stress Biomarkers

Oxidative stress biomarkers were evaluated to elucidate the protective mechanism of flavonoid treatment. DMN exposure significantly reduced antioxidant enzyme activities, including SOD and CAT, while markedly increasing MDA levels, indicating excessive lipid peroxidation and oxidative damage. These findings are consistent with previous reports identifying oxidative stress as a central mechanism in DMN-induced hepatic injury (Haenen & Bast, 1983; Liu et al., 2023). Treatment with *Pometia pinnata* flavonoids significantly restored antioxidant enzyme activity and suppressed lipid peroxidation in a dose-dependent manner, with the high-dose group showing the most pronounced effect. Similar antioxidant restoration by flavonoids has been widely reported and is attributed to their ability to scavenge reactive oxygen species and activate Nrf2-mediated antioxidant pathways (Wongkajornsilp et al., 2020; Khan et al., 2023; Swilam et al., 2022).

DISCUSSION

The findings of this study provide compelling evidence that the investigated herbal formulation exerts meaningful antidiabetic effects through a multi-mechanistic mode of action. The integration of LC-HRMS metabolomic profiling, in vitro cellular evaluation, and in silico molecular docking enabled a comprehensive interpretation of biochemical interactions, functional bioactivity, and predicted target engagement. The combination of these analytical platforms strengthened the biological relevance of the results and supported the hypothesis that the formulation acts as a multi-target therapeutic rather than a single-compound intervention.

Metabolomic analysis revealed a diverse chemical profile dominated by phenolic acids, flavonoids, and alkaloids, compound classes that have been extensively associated with improvements in oxidative balance, insulin sensitivity, and glucose metabolism. Several identified metabolites, including quercetin derivatives, gallic acid, and ellagic acid, have been reported to exert antidiabetic effects by enhancing mitochondrial stability, reducing reactive oxygen species

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formation, and modulating key metabolic pathways involved in insulin resistance (Liu et al., 2021; Zhang et al., 2020). LC-MS-based studies further demonstrate that extracts containing multiple phenolic constituents exhibit stronger antidiabetic efficacy than isolated compounds, supporting the concept of synergistic interactions among phytochemicals (Alam et al., 2022). Moreover, integrated metabolomics and molecular docking analyses have identified these compounds as high-affinity ligands for diabetes-related targets such as α-glucosidase and AMPK, reinforcing their functional relevance (Lekmine et al., 2023). Collectively, the chemical diversity detected through LC-HRMS suggests that the therapeutic potential arises from multi-component synergy rather than the action of a single dominant constituent.

Biological evaluation using INS-1 pancreatic β-cells demonstrated that treatment with the extract improved cell viability and glucose-stimulated insulin secretion. This functional enhancement suggests protection against β-cell dysfunction, which is a critical pathological event in the progression of type 2 diabetes mellitus. The observed increase in antioxidant capacity and reduction of intracellular oxidative stress support the hypothesis that the extract attenuates cellular injury induced by reactive oxygen species. The dose-dependent pattern observed in the results further implies that bioactivity correlates with metabolite concentration and that the formulation maintains efficacy within a safe exposure range. This behavior is consistent with the pharmacological characteristics of polyphenolic bioactives known to exert hormetic effects.

Molecular docking analysis provided mechanistic insight into how the identified metabolites may interact with diabetes-related molecular targets. The strong predicted binding affinities with enzymes such as α-glucosidase and α-amylase indicate potential inhibitory activity that could contribute to delayed carbohydrate digestion and postprandial glucose reduction. In addition, the molecular interaction patterns with regulatory proteins involved in the PI3K-AKT and AMPK pathways suggest a plausible role in enhancing insulin signaling and glucose uptake. Notably, several compounds particularly quercetin derivatives, gallic acid, and ellagic acid exhibited high-affinity interactions with multiple protein targets involved in oxidative stress regulation, glucose metabolism, and inflammatory signaling, supporting a network-based pharmacological mechanism rather than a single-target mode of action.

The correlation between metabolomics data, biological responses, and computational predictions highlights a clear relationship between specific chemical constituents and measurable functional benefits. The improvement in biochemical markers and the convergence of multi-target interactions indicate that the extract operates through both direct and indirect mechanisms. The biological improvements observed in comparison to the untreated diabetic model suggest not only prevention of cellular damage but also restoration of metabolic integrity. These findings are consistent with previous metabolomics-driven pharmacological studies demonstrating that polyphenol-rich extracts exert therapeutic effects through multi-target mechanisms involving enzyme inhibition, antioxidant defense, and signaling modulation (Hopkins, 2008; Johnson et al., 2016; Zhang et al., 2019).

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The results support the conclusion that the antidiabetic activity of the herbal formulation results from a combination of enzyme inhibition, antioxidant protection, modulation of signaling pathways, and enhancement of pancreatic function. The multidisciplinary approach employed in this study provides a strong scientific foundation for further exploration and potential development into standardized therapeutic applications. Future work should focus on transcriptome-level validation, in vivo pharmacodynamic and pharmacokinetic profiling, and identification of metabolite-target interactions in physiological conditions to confirm the mechanisms proposed in this study. A larger sample size and clinical translational research would further strengthen the applicability and therapeutic value of this formulation.

CONCLUSIONS

The findings of this study demonstrate that the herbal formulation exhibits significant antidiabetic potential through a synergistic, multi-target mechanism. The LC-HRMS metabolomics profiling revealed diverse bioactive constituents including flavonoids, phenolic acids, and alkaloids, which are strongly associated with antioxidant and glucose-regulating activities. The in vitro biological evaluation confirmed that the extract enhanced glucose-stimulated insulin secretion and improved β-cell viability, suggesting that it offers cytoprotective benefits under diabetic stress conditions. Molecular docking analysis further supported these observations by demonstrating strong binding affinities between key metabolites and diabetes-related protein targets, including α-glucosidase, α-amylase, AMPK, IRS-1, PI3K, and GLUT4 regulatory complexes.

Collectively, these findings indicate that the antidiabetic activity of the formulation is not the result of a single compound but rather the cumulative action of multiple phytochemicals working in a coordinated manner. The integration of computational, analytical, and biological datasets provides strong evidence that the extract may modulate insulin signaling pathways, improve glucose uptake, and inhibit carbohydrate-digesting enzymes. Although the results are promising, further studies including transcriptomic validation, pharmacokinetic profiling, and clinical evaluation are required to confirm these mechanistic insights and assess long-term safety and efficacy. Overall, this study provides a scientific foundation supporting the traditional therapeutic use of the formulation and highlights its potential as a complementary or alternative antidiabetic treatment candidate.

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