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RESEARCH ARTICLE

# Antidiabetic Activity of Flavonoid Extract from *Wattakaka volubilis* Linn. *In Silico* and *In Vivo* Approaches

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## Abstract

**Background:** Diabetes mellitus is a chronic metabolic disorder characterised by persistent hyperglycemia and impaired metabolism of carbohydrates, fats, and proteins. *Wattakaka volubilis* Linn. has been widely used in the traditional Indian medicine system. **Aim:** The present study aimed to evaluate the antidiabetic efficacy of an ethanolic extract of *W. volubilis* leaves in alloxan-induced diabetic rats, supported by *in silico* molecular docking studies. **Methods:** The plant extract was prepared by shade-drying the leaves, grinding them into a fine powder, and extracting the powdered leaves sequentially with hexane, chloroform, and ethanol using a Soxhlet apparatus. Phytochemical screening and Thin-Layer Chromatography (TLC) were performed to identify bioactive compounds with antidiabetic potential. An *in silico* approach was employed to assess the efficacy of flavonoid compounds from *Wattakaka volubilis* Linn. as antidiabetic agents. The three-dimensional structure of the insulin receptor was downloaded from the Protein Data Bank under the accession number 1IR3. Flavonoid compounds, which include isoflavones and flavanones, were downloaded from the PubChem database. The studies on docking interactions between the insulin receptor and the bioactive compounds from the plant were carried out using the Schrodinger software. Diabetes was created in rats by giving them a single intraperitoneal injection of alloxan monohydrate (150 mg/kg), and the animals were checked for hyperglycemia. **Results:** The subsequent tests tested the antidiabetic activities of the ethanolic extract. Docking analysis showed that eriodictyol and hesperetin had good binding interactions; eriodictyol had a binding energy of -5.91 kcal/mol, indicating a high possibility of activating the insulin receptor. **Conclusion:** The findings from both *in vivo* and *in silico* studies indicate the therapeutic potential of *W. volubilis* Linn. in diabetes management and call for further research.

**Major Findings:** The ethanolic extract of *Wattakaka volubilis* Linn. showed significant antidiabetic activity mainly due to the presence of *eriodictyol* as an active constituent.

**Keywords:** Diabetes, Ethanol Extract, Eriodictyol, Flavonoids, Insulin Receptor, Molecular Docking, *Wattakaka volubilis*

## 1. Introduction

Diabetes is among the world's oldest known diseases and affects millions globally[1]. Current estimates indicate a worldwide diabetes prevalence of approximately 4%, impacting around 143 million people — a number projected to reach 300 million by 2025[2]. In 2021, approximately 10.5% of individuals aged 20-79 worldwide were living with diabetes, which translates to about 536.6 million people. This figure is projected to increase to 12.2% (783.2 million people) by 2045. Diabetes impacts both men and women equally, with the highest prevalence found in those aged 75-79. It is more common in urban settings (12.1%) compared to rural areas (8.3%), and in high-income countries (11.1%) compared to low-income countries (5.5%). The greatest increase in diabetes rates from 2021 to 2045 is expected in middle-income countries (21.1%),

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followed by high-income nations (12.2%) and low-income nations (11.9%). In 2021, global healthcare spending related to diabetes was estimated at 966 billion USD, and this is expected to rise to 1,054 billion USD by 2045[3]. Despite the availability of various oral hypoglycemic agents and insulin therapies, an effective cure for diabetes remains elusive[4]. Diabetes-related metabolic abnormalities primarily impact key tissues like adipose tissue, skeletal muscle, and the liver due to insulin resistance. The intensity of symptoms depends on the type and duration of diabetes. Individuals with significantly elevated blood sugar levels, particularly those with an absolute insulin deficiency (as seen in children with Type 1 diabetes), may present with symptoms such as increased hunger, excessive thirst (polydipsia), frequent urination (dysuria), unexplained weight loss, and vision disturbances. However, many individuals, especially those with early-stage Type 2 diabetes, may remain asymptomatic, making early detection challenging[5].

In recent years, herbal remedies have gained global attention due to their perceived therapeutic efficacy and safety. Numerous traditional medicinal plants have been evaluated for their antidiabetic properties in experimental animal studies[6]. Phytochemicals obtained from these plants are known for their role in preventing and treating various diseases. They are found affordable, effective, and firmly rooted in traditional healing traditions across the globe, to therefore can be relied upon for healthcare wise[7]. A 2008 report by the World Health Organisation (WHO) estimated that around 80% of diabetic patients rely on herbal medicine for effective management of their condition. Medicinal phytochemicals are commonly used either on their own or alongside antidiabetic drugs, offering a complementary approach to treatment[8].

*In-silico* research plays a crucial role in drug development by streamlining the identification, design, and evaluation of potential drug candidates. It enables early toxicity prediction, helping researchers avoid harmful compounds and save time and resources. Techniques like molecular modelling and virtual screening predict binding modes and interactions, leading to the discovery of novel therapies. This approach has already contributed to breakthroughs, such as the development of HIV treatment raltegravir, melanoma drug vemurafenib, and COVID-19 treatment molnupiravir. *In-silico* drug discovery has become a significant industry, valued at $8.3 billion in 2022, with continued growth expected[9]. In this study, we used an *in-silico* approach to examine flavonoid compounds from the nutritionally enriched plant *W. volubilis*, which show potential as activators of insulin receptor tyrosine kinase, potentially helping to mitigate the effects of diabetes. Insulin binds to receptors on various body cells to facilitate glucose absorption, with these receptors belonging to the tyrosine kinase class[10]. Computational biology and bioinformatics are crucial tools in modern drug design and screening. By using molecular docking, we gained valuable insights into the binding interactions and affinity between drug molecules and their target receptors. Specifically, our bioinformatics and molecular docking analyses highlighted the potential of flavonoids, a compound present in *W. volubilis*, for managing Diabetes Mellitus (DM). Encouraged by these findings, we proceeded to isolate and purify flavonoids from *W. volubilis* leaf extract. Subsequently, we tested the purified compound through *in vivo* experiments in an animal model of diabetes to evaluate its therapeutic efficacy[11].

Traditionally, *W. volubilis*, belonging to the *Asclepiadaceae* family, has been used in Indian medicine for various therapeutic purposes[12]. The leaves are applied to relieve rheumatic pain, cough, fever, and severe cold. Preparations containing its bark and leaf powder have been used for treating urinary problems and diabetes, respectively[13]. Although *W. volubilis* has gained significant attention in the traditional medicinal field, there is a scarcity of research on the scientific validation of its bioactive compounds as well as their mechanisms of action. Although flavonoids are identified as the potential antidiabetic drugs, their exact binding interaction and pharmacological role in *W. volubilis* have not been investigated further. Most of the research done on herbal remedies is based on traditional uses with limited integration of molecular-level mechanisms and experimental evidence. This gap calls for a multidisciplinary approach that integrates *in silico, in vitro*, and *in vivo* studies to explore the therapeutic efficacy of *W. volubilis*. Based on these conventional uses and initial *in silico* findings, the present study investigates the antidiabetic potential of an ethanolic leaf extract of *W. volubilis* Linn. in rats that were rendered diabetic with alloxan.

## 2. Materials and Methods

### 2.1 Plant Material

*Wattakaka volubilis* Linn. leaves were freshly collected from Madurai, located in the southern Ghats of Tamil Nadu, India. The plant was identified and authenticated by the Botanical Survey of India, Southern Circle, Madurai, Tamil Nadu, with a voucher specimen deposited in the Ethnopharmacology Unit, Research Department of Botany, at American College, Madurai, Tamil Nadu.

### 2.2 Preparation of Plant Extract for Phytochemical Screening and Antidiabetic Studies

The leaves of *W. volubilis* were shade-dried at room temperature and ground into a fine powder using a Wiley mill. For extraction, 100 grams of the powdered leaves were packed into a *Soxhlet* apparatus and sequentially extracted with hexane, chloroform, and ethanol. Phytochemical screening was performed using standard methods to identify the presence of various compounds[14]. An alkaline reagent test confirmed the presence of flavonoids, specifically isoflavones and flavanones. The ethanol extract was then concentrated using a rotary evaporator, and the resulting concentrate was used for the antidiabetic studies.

### 2.3 Protein Structure and Ligand Preparation

The 3D structure of the insulin receptor protein complexed with its peptide substrate was obtained from the PDB (Protein Data Bank) database. Ligand structures in CID format were retrieved from the PubChem database (http://pubchem.ncbi.nlm.nih.gov/), a repository of chemical molecules. Using Open Babel (http://www.openbabel.org/), these structures were converted to PDB format, suitable for docking studies. Ligands were selected based on Lipinski's rule of five to ensure drug-likeness[15].

#### *2.3.1 Active Site Analysis*

Validating the binding site is a critical step in molecular docking as it identifies ligand-binding regions, key amino acid residues, and site scores. Using the SiteMap tool in Maestro (version 10.2, Schrödinger suite), we analysed and generated a comprehensive map of the protein's binding sites. Among the identified sites, we selected one based on its site score, prioritising the site with the largest volume for grid generation.

Grid generation is a crucial step in defining the target site on protein molecules for docking studies. Using grid-based ligand docking, phytocompounds or commercial drugs were docked with the protein target to evaluate key docking parameters. A grid box was created, centred on the target site, to ensure precise docking of the ligand.

#### *2.3.2 Docking Studies*

Molecular docking was conducted using flexible docking to predict binding affinity, ligand efficiency, and inhibitory constants. All ligands were docked at the target's active site using the Glide Extra Precision (XP) module. Only active compounds achieved optimal positioning, avoiding penalties and securing favourable scores by forming accurate hydrophobic interactions between the protein and ligand.

### 2.4 Animals

The study used healthy male Wistar albino rats, each weighing between 180-240 g. The animals were housed in standard conditions with a temperature of 25±2°C, a 12-hour light/dark cycle, and provided with a standard pellet diet (Goldmohur brand, MS Hindustan Lever Ltd., Mumbai, India) and water ad libitum.

#### *2.4.1 Acute Toxicity Study*

Acute oral toxicity was evaluated according to OECD-423 guidelines (acute toxic class method). Six randomly selected albino rats were fasted overnight and then administered the plant extracts by gastric intubation at an initial dose of 5 mg/kg body weight. The animals were monitored for 14 days for any signs of toxicity or mortality. If mortality occurred in two of the three animals, the dose was classified as toxic. If mortality was observed in one animal, the dose was retested. This procedure was repeated with increasing doses (50, 100, and 1000 mg/kg) until the toxic dose was determined.

#### *2.4.2 Induction of Experimental Diabetes*

Diabetes was induced in the rats by administering a single intraperitoneal injection of alloxan monohydrate (150 mg/kg). Two days after injection, rats with blood glucose levels between 200-260 mg/dL, as confirmed

by glycosuria and hypoglycemia, were selected for the study. These rats had free access to water and food and were kept at room temperature in plastic cages.

#### 2.4.3 Experimental Design

A total of 24 rats (18 diabetic and 6 normal) were divided into four groups of six rats each:

Group I: Normal, untreated rats

Group II: Diabetic control rats

Group III: Diabetic rats treated with *Wattakaka volubilis* leaf ethanol extract (100 mg/kg body weight)

The dosage of 100 mg/kg was selected based on previous studies that have demonstrated significant antidiabetic activity at this dose in rodent models[16,17].

Group IV: Diabetic rats treated with the standard drug glibenclamide (600 µg/kg body weight)

#### 2.4.4 Biochemical Analysis

At the end of the 14-day experimental period, the animals were sacrificed by decapitation. Blood samples were collected, and serum was separated by centrifugation at 3000g for 10 minutes. Serum glucose levels were measured using the O-toluidine method, while insulin levels were assayed using an ELISA kit.

### 2.5 Statistical Analysis

Data were analysed using the Student's t-test. Statistical significance was set at p-values of less than 0.01 and 0.05.

## 3. Results

### 3.1 Qualitative Analysis of Phytochemicals

Phytochemical analysis of the ethanol extract from *W. volubilis* leaves revealed the presence of various bioactive compounds, including alkaloids, coumarins, flavonoids, glycosides, terpenoids, tannins, phenols, saponins, and steroids. Research has shown that flavonoids can mimic insulin, affecting several signalling mechanisms involved in glucose uptake and utilisation[18]. Active isoflavones and flavanones identified are listed in Table 1, based on data from the USDA Database.

### 3.2 Molecular Properties and Ligand Selection

The 3D structure of the human insulin receptor (PDB ID: 1IR3) was retrieved from the RSCB Protein Databank, featuring 546 residues, 3,159 atoms, 45 heteroatoms, and 202 solvent atoms, and was selected for docking analysis. Mol Inspiration, an online tool, was used to identify essential molecular properties of selected ligands, such as logP, molecular weight, hydrogen bond donors, and hydrogen bond acceptors. These properties were then analysed according to Lipinski's Rule of Five, which is used to determine drug-likeness. Based on this analysis, probable ligands, including daidzein, glycetein, hesperetin, naringenin, eriodictyol, homoeriodictyol, and genistein, were selected. The filter values for these compounds are provided in Table 2.

#### 3.2.1 Binding Sites and Docking Studies

The catalytic pockets of the target protein, human insulin receptor, were analysed using the SiteMap tool.

**Table 1.** Identified active constituents in *W. volublis*

| S. No. | ISO Flavones | Flavonones |
|---|---|---|
| 1 | Daidzein | Hesperetin |
| 2 | Genistein | Naringenin |
| 3 | Glycitein | Eriodictyol |
| 4 | | Homo eriodictyol |

**Table 2.** The Lipinski Filter Values of compounds

| Compound | Compound ID | Mol. weight g/mol) | Mol. formula | Site score | H-bond donor | H-bond acceptor | Molare refaractivity |
|---|---|---|---|---|---|---|---|
| Glycitein | **5317750** | 254.00 | C16H12O5 | 2.71 | 2 | 4 | 69.14 |
| Daidzein | 5281708 | 284.00 | C15H10O4 | 2.72 | 2 | 5 | 75.70 |
| Genistein | 5250961 | 270.00 | C15H10O5 | 2.42 | 3 | 5 | 70.81 |
| Hesperetin | 72281 | 302.27 | C16H14O6 | 2.40 | 3 | 6 | 71.69 |
| Naringenin | 439246 | 272.00 | C15H12O5 | 2.51 | 3 | 2 | 66.32 |
| Eriodictyol | 440735 | 302.00 | C15H12O6 | 2.52 | 3 | 6 | 71.69 |
| Homoeriodictyol | 73635 | 288.00 | C16H14O6 | 2.21 | 4 | 6 | 67.16 |

Protein preparation involved the Protein Preparation Wizard, where non-hydrogen-bonded crystallographic water molecules were removed. Missing side-chain atoms were added, and structural breaks were repaired using Prime. Hydrogen bonds were optimised to reflect a pH of 7.0, considering appropriate ionisation states of acidic and basic amino acid residues. The catalytic site of the human insulin receptor was predicted using the CASTp program, which accurately calculated the area and volume of each pocket. The most favourable binding site was identified as pocket 36, with a volume of 1178.9 $Å^3$ and an area of 901.1 $Å^2$, comprising 36 amino acid residues, including Met343, Leu346, Thr347, and Glu353. The same binding site and amino acid residues were observed in this study.

Docking program validation was performed by calculating the Root-Mean-Square Deviation (RMSD) between the co-crystallised pose of the native ligand and its redocked pose. The RMSD was 0.8205 Å, confirming accurate reproduction of the ligand's position within the binding domain. This aligns with the standard that RMSD values should not exceed 2 Å for reliable docking validation. Molecular docking studies explored the binding interactions between insulin receptor and the daidzein, glycetein, hesperetin, naringenin, eriodictyol, homoeriodictyol, and genistein, alongside the native ligand and reference drugs glibenclamide. The binding affinities of the designed compounds were evaluated using G-scores, which ranged from −5 kcal/mol to −43.2 kcal/mol, indicating strong interactions with the target receptor (Table 3). Among them, eriodictyol achieved the highest binding affinity, with a G-score of −14.2 kcal/mol. The Ramachandran plot of the insulin receptor confirmed that all residues were within the favoured regions (Figure 1).

Daidzein showed a binding energy of -12.16 kcal/mol and interacted with residues GLU990, SER1067, LEU1062, THR1072, ASN1046, PHE1151, and ASP1150 through hydrogen bonds. *Glycetein* had a binding energy of -17.20 kcal/mol and interacted with GLU990, SER1067, LEU1062, GLU1047, ASN1046, ILE1148, PHE1154, GLU1043, ARG1039 via hydrogen bonding. *Genistein* displayed a binding energy of -23.41 kcal/mol, forming hydrogen bonds with residues TRP 989, GLU 990, LEU 1062, VAL 1065, SER 1067, LEU 1045, VAL 1059, PHE 1151, THR 1154, and LEU 1133. Hesperetin demonstrated the strongest binding energy at -25.66 kcal/mol, interacting with LEU1171, LEU1170, PHE1151, ILE1148, VAL1059, GLU1047 SER 1006, PHE 1007, ASP1132, ARG1131. In contrast (Figure 3), Eriodictyol had a strong binding energy of -26.37 kcal/mol, forming hydrogen bonds with residues LEU1170, GLU988, ILE1062, ARG1131, GLU1047, LEU1148, VAL1059, GLY1152, CYS1138, GLU1043 PTR1163, SER1006, PHE1007 (Figure 2).

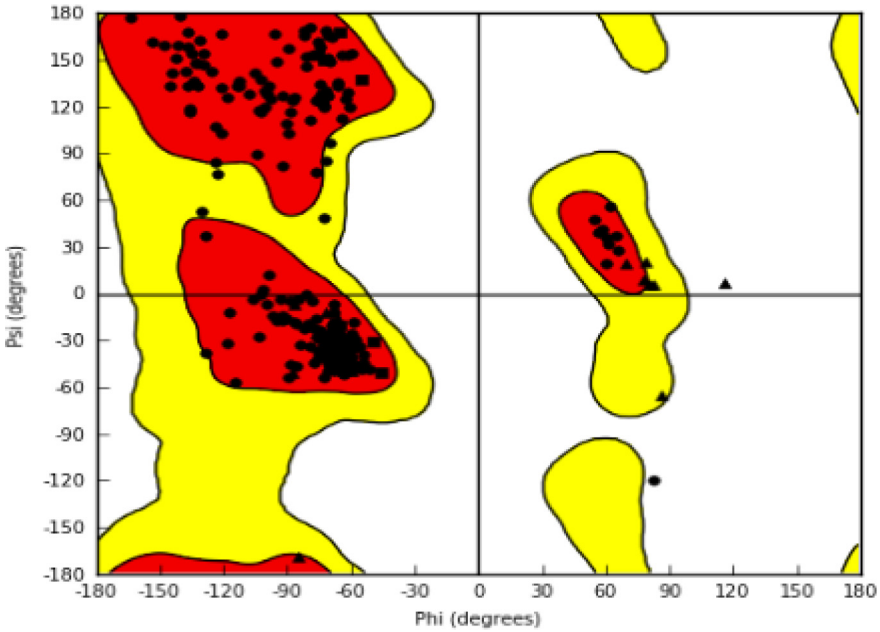



**Figure 1.** Ramachandran plot.

**Table 3.** The results of docked molecules and binding sites of molecules

| S. No. | Protein | Drug (Ligand) | Energy range: Emin | Energy range: Emax | Binding site residues |
|---|---|---|---|---|---|
| 1 | 1IR3 | Daidzein | -12.16 | -36.39 | GLU990, SER1067, LEU1062, THR1072, ASN1046, PHE1151, ASP1150 |
| 2 | 1IR3 | Glycetein | -17.20 | -40.37 | GLU990, SER1067, LEU1062, GLU1047, ASN1046, ILE1148, PHE1154, GLU1043, ARG1039 |
| 3 | 1IR3 | Genistein | -23.41 | -37.77 | TRP989, GLU990, LEU1062, VAL1065, LEU1045, VAL109, PHE1151, THR11154, LEU1133 |
| 4 | 1IR3 | Hesperidins | -25.66 | -43.14 | LEU1171, LEU1170, PHE1151, ILE1148, VAL1059, GLU1047 SER 1006, PHE 1007, ASP1132, ARG1131 |
| 5 | 1IR3 | Naringenin | -21.83 | -30.60 | LEU1170, LEU1062, ARG1061, GLY1064, GLU1047, PHE1 151, VAL1059, GLY1149, HIS1130, PHE1151, LEU1135 |
| 6 | 1IR3 | Eriodictyol | -26.37 | -43.09 | LEU1170, GLU988, ILE1062, ARG1131, GLU1047, LEU1148, VAL1059, GLY1152, CYS1138, GLU1043 PTR1163, SER1006, PHE1007 |

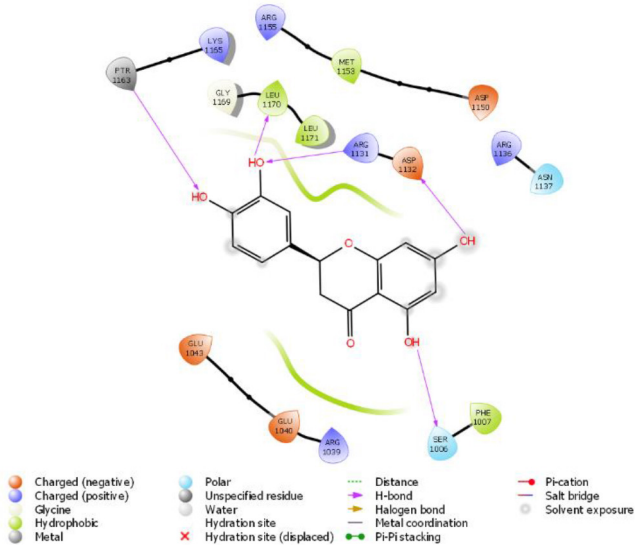



**Figure 2.** 2D interaction of ligand in the active site of 1IR3 insulin receptor tyrosin kinase activator440735.

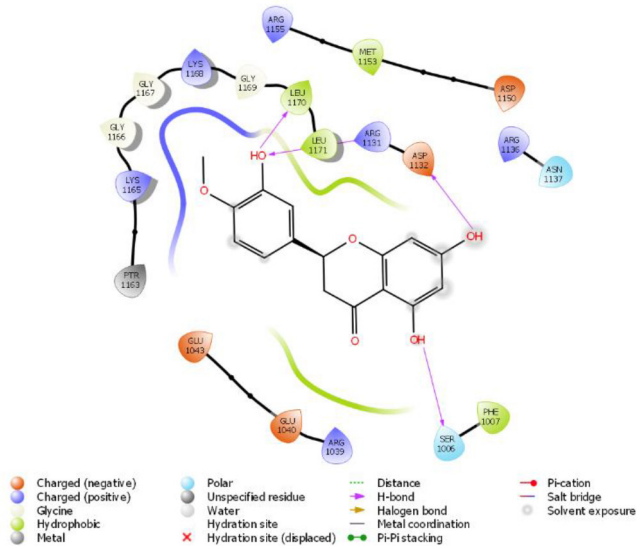



**Figure 3.** 2D interaction of ligand in the active site of 1IR3 insulin receptor tyrosin kinase activator72281.

## 3.3 Biological Activity

### *3.3.1 Acute Toxicity and Antidiabetic Activity*

The acute toxicity study confirmed the ethanol extract of *Wattakaka volubilis* leaves to be non-toxic. In alloxan-induced diabetic rats, a significant increase in blood glucose levels (from 75.31 to 225.34 mg/dL, $p<0.01$) and a substantial decrease in plasma insulin levels (from 19.51 to 4.31 µU/mL, $p<0.01$) were observed. However, treatment with *Wattakaka volubilis* ethanol extract significantly reduced blood glucose levels and increased plasma insulin levels at a dose of 150 mg/kg body weight (Table 4).

**Table 4.** Effect of ethanol extract of *Wattakaka volubilis* leaf on serum glucose of normal, diabetic induced and drug-treated rats

| S. No. | Parameter | Glucose (mg/dl) | Insulin (µg/dl) |
|---|---|---|---|
| 1 | **GroupI** | 78.31±5.12 | 16.51±0.66 |
| 2 | **GroupII** | 215.34±6.31** | 4.11±0.36** |
| 3 | **GroupIII** | 106.14±3.81 | 10.21±2.31 |
| 4 | **GroupIV** | 101.21±1.42[a] | 15.32±2.42 |

Each value is the SEM of animals' comparison was made between normal control to diabetic control, and drug treated *$p<0.05$; **$p<0.01$ and comparisons were

made between diabetic control to drug treated groups: a $p<0.05$ level.*$p<0.05$; **$p<0.01$ and comparisons were made between diabetic control to drug treated groups: [a] $p<0.05$level.

## 4. Discussion

The present study investigates the bioactive potential of *W. volubilis* leaves, focusing on its phytochemical composition, molecular interaction with the human insulin receptor, and its effect on blood glucose regulation. The findings indicate that the ethanol extract of *W. volubilis* contains a variety of bioactive compounds with potential therapeutic implications, particularly in the context of diabetes management. These results are discussed in light of current literature on phytochemicals, their molecular mechanisms, and their biological activities, especially antidiabetic effects.

### 4.1 Phytochemical Composition and Potential Antidiabetic Properties

The qualitative phytochemical analysis of *W. volubilis* leaves revealed the presence of several bioactive compounds, including alkaloids, coumarins, flavonoids, glycosides, terpenoids, tannins, phenols, saponins, and steroids. Among these, flavonoids are of particular interest due to their known role in glucose homeostasis. Isoflavones such as daidzein, genistein, glycetein, and flavanones like hesperetin, naringenin, and eriodictyol have demonstrated insulin-mimetic and glucose-lowering effects in various studies. For example, genistein and daidzein are known to activate insulin signalling pathways by enhancing glucose uptake and improving insulin sensitivity in adipocytes and skeletal muscle cells[18,19]. Similarly, naringenin has been shown to improve insulin secretion and enhance glucose metabolism by modulating the PI3K/Akt signalling pathway[20].

The presence of these bioactive flavonoids in the extract of *W. volubilis* supports its potential use in the management of diabetes. The identification of these compounds is consistent with previous reports on other plant species with similar medicinal applications[21]. However, the specific contribution of each of these compounds to the observed biological effects of the plant extract requires further investigation, particularly through the use of purified compounds in *in vitro* and *in vivo* models.

### 4.2 Molecular Docking and Binding Affinity to Human Insulin Receptor

To explore the molecular mechanisms underlying the antidiabetic effects of *W. volubilis* leaves, the molecular docking studies were conducted with key flavonoids, including daidzein, glycetein, hesperetin, naringenin, eriodictyol, homoeriodictyol, and genistein, and the human insulin receptor (PDB ID: 1IR3). The docking studies revealed that these compounds interacted strongly with the catalytic site of the insulin receptor, demonstrating promising binding affinities. Among the compounds, eriodictyol exhibited the strongest binding energy of −26.37 kcal/mol, followed closely by hesperetin (−25.66 kcal/mol), suggesting that these flavonoids may have the highest potential for interacting with the insulin receptor and modulating its activity.

The binding sites identified in this study corresponded with known functional residues within the insulin receptor, such as GLU990, SER1067, and PHE1151, which are critical for the receptor's ligand binding and activation[22]. The favourable binding of flavonoids like eriodictyol and hesperetin with these residues indicates that these compounds may mimic insulin and enhance receptor activation, thereby improving glucose uptake[23]. These findings align with previous molecular docking studies, which demonstrated the potential of flavonoids as insulin receptor modulators.

Moreover, the validation of the docking protocol, with an RMSD value of 0.8205 Å between the co-crystallised and redocked poses, further supports the reliability of the docking results. The observed binding energies of the flavonoids, ranging from −12.16 to −26.37 kcal/mol, suggest a strong potential for these compounds to modulate insulin receptor activity, reinforcing the hypothesis that *W. volubilis* may offer therapeutic benefits for diabetes management through receptor-mediated mechanisms.

### 4.3 Biological Activity: Acute Toxicity and Antidiabetic Effects

The acute toxicity study demonstrated that the ethanol extract of *W. volubilis* leaves is non-toxic, with no observed adverse effects in experimental animals. This is a promising result, indicating the safety of the extract for potential therapeutic use. In the alloxan-induced

diabetic rat model, the ethanol extract significantly reduced blood glucose levels and increased plasma insulin levels at a dose of 150 mg/kg body weight. This dose was effective in reversing the diabetes-induced hyperglycemia and hypoinsulinemia, which are characteristic of this model. The results showed a significant reduction in blood glucose levels (from 225.34 mg/dL to 106.14 mg/dL) and a notable increase in plasma insulin levels (from 4.11 µU/mL to 10.21 µU/mL), suggesting that the extract has both insulinotropic and hypoglycemic effects.

The glucose-lowering effect of *W. volubilis* extract is consistent with the observed binding affinities of the flavonoids in the docking studies. It is plausible that the flavonoids in the extract act through insulin receptor modulation, promoting glucose uptake and reducing hyperglycemia. Additionally, the insulinotropic effect of the extract may be attributed to the presence of compounds that influence β-cell function or enhance insulin secretion, as demonstrated for other flavonoid-rich plant extracts.

### 4.4 Implications and Future Directions

The findings from this study suggest that *W. volubilis* leaves contain a promising array of flavonoids with the potential to modulate insulin receptor activity and improve glucose homeostasis. These results provide a strong rationale for further investigation of *W. volubilis* as a source of natural compounds for the management of diabetes. Future studies should focus on isolating and characterising individual bioactive compounds, exploring their effects on insulin signalling pathways, and evaluating their efficacy in clinical trials.

Additionally, the mechanisms underlying the observed biological effects, including the influence of flavonoids on insulin secretion and β-cell function, should be further explored using in vitro models, such as pancreatic β-cell lines and primary islet cultures. Furthermore, long-term toxicity studies and pharmacokinetic evaluations will be necessary to assess the safety and bioavailability of the active compounds.

## 5. Conclusion

Molecular docking plays a crucial role in drug discovery and development, with *in silico* approaches allowing us to assess the potential of nutritionally rich plants like *W. volubilis* as sources of antidiabetic agents. This study demonstrated that flavonoid compounds from *W. volubilis*, particularly in the flavanone subgroup, may serve as insulin receptor tyrosine kinase activators, helping to mitigate the adverse effects of diabetes. Among these, Eriodictyol emerged as the most active compound, showing the highest binding energy (-26.37 kcal/mol) and binding to key receptor sites (SER1006, LEU 1170, GLU 988, and PHE 1007) through hydrogen bonding. This interaction suggests that Eriodictyol could be a potent inhibitor of the 1IR3 receptor, positioning it as a primary antidiabetic compound.

The findings suggest that the ethanolic extract of *W. volubilis* leaves, rich in phytochemicals such as flavonoids, terpenoids, tannins, glycosides, sterols, phenols, and saponins, holds significant antidiabetic potential. Flavonoids, sterols/terpenoids, and phenolic acids are well-documented as bioactive agents with antidiabetic properties. Flavonoids, in particular, are noted for their ability to regenerate damaged beta cells in diabetic models, while phenolics are valued for their antihyperglycemic effects. The antidiabetic activity of the *W. volubilis* leaf extract is likely due to the synergistic action of these multiple bioactive compounds. This study provides valuable insights into the mechanisms behind the plant's antidiabetic effects. These findings support further studies on *W. volubilis* flavonoids as potential antidiabetic agents, warranting clinical validation.

## 6. Authors Contribution:

Conceptualization, J.B.A.J.; methodology, J.B.A.J.; Lab work, J.B.A.J. and S.J.; writing—original draft preparation, J.B.A.J. and S.J.; writing—review and editing, J.B.A.J. and S.J.; visualization, J.B.A.J. and S.J.; supervision, J.B.A.J. and S.J. All authors have read and agreed to the published version of the manuscript.

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