ANTIOXIDANT PROPERTIES AND ANTI-DIABETIC EFFICACY OF A POLYHERBAL FORMULATION ADVOCATED IN THE SOUTHWEST OF ALGERIA FOR THE MANAGEMENT OF DIABETES: *IN VITRO*, *IN VIVO*, AND MOLECULAR DOCKING INVESTIGATIONS

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ABSTRACT

Diabetes mellitus is a leading cause of worldwide mortality, and current diabetes medications have serious adverse effects. Therefore, herbal medicine is gaining popularity as a complementary treatment for diabetes. This study investigated the polyphenolic content and antioxidant activity of a polyherbal formulation (PHF), which is used in traditional medicine in the Adrar region (Southwestern Algeria) for the management of diabetes mellitus. We also investigated the anti-diabetic activity of infusions from PHF (*i.e*., PHFI, 0.5%, and 1%) and PHF-based diets (*i.e*., PHFD, 25% and 50%) in streptozotocin-induced diabetic rats. Thirty male Wistar rats were used in this study and randomly divided into six groups (n=5 per group) using a completely randomized design (CRD). Diabetes was induced by a single (i.p) injection of streptozotocin (60 mg/kg), followed by oral administration of either PHF infusions or diets for 28 days. A molecular docking analysis using AutoDock Vina was conducted to predict the binding affinity of *p*-Coumaric acid (*p*-CA), the most dominant phenolic compound found in the main PHF ingredients towards four diabetes-related target proteins (AMPK, LKB1, ChREBP, and TBC1D1). Phytochemical assessment revealed the abundance of tannins, flavonoids, and polyphenols (0.87 mg CE/100 g DW, 0.82 mg CE/g DW, and 1.43 mg GAE/g DW, respectively) in the crude extract of the PHF. *In vitro* DPPH, FRAP, and ABTS assays revealed that the crude extract of PHF possesses significant antioxidant activity against all tested radicals, with an IC_{50} of 0.025 mg/mL for DPPH. Most experimental groups (*i.e*., PHFD, 25%; PHFD, 50%; and PHFI, 1%) demonstrated a significant ($P < 0.05$) improvement in glycemia, corticosterone levels, hepatic biomarkers, and lipid profile (HDL, LDL, TG, and TC). *In silico* results indicate that *p*-CA demonstrates superior binding affinities and interactions with the target proteins compared to metformin, with binding affinities of −6.6, −5.6, −6.5, and −5.5 kcal/mol for AMPK, LKB1, ChREBP, and TBC1D1, respectively. The considerable anti-hyperlipidemic, antioxidant, and anti-diabetic activities of the studied FPH validate its application in complementary and alternative medicine for diabetes management.

Keywords: Diabetes mellitus, Wistar rat, polyherbal formulation (PHF), antioxidant, molecular docking

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INTRODUCTION

Diabetes mellitus (DM), or commonly diabetes, represents a long-term hormonal and metabolic disorder marked by persistent hyperglycemia due to either impaired insulin production, peripheral tissue resistance, or both (ElSayed *et al.*, 2023). Besides, these physiological changes are associated with alterations in protein and lipid metabolism in peripheral tissues (Stojkovic *et al.*, 2019). Chronic hyperglycemia can result in several expensive and dangerous health problems affecting both smaller and larger vessels, some of which can be fatal (ElSayed *et al.*, 2023). If untreated, diabetes can lead to several diseases and elevate the risk of severe microvascular complications, including diabetic retinopathy, nephropathy as well as macrovascular complications, such as heart failure, peripheral artery disease, and coronary heart disease (Tomic *et al.*, 2022).

DM is one of the significant health issues that has become a global epidemic in the twenty-first century (Standl *et al.*, 2019). In this context, the global prevalence of DM has been rising rapidly in recent years, and more rapidly (80.63%, 474.7 million) in low- and middle-income countries than in high-income countries. Indeed, approximately 589 million adults had diabetes in 2024, or 11.1% of the world's adults, with future predictions indicating an elevation to 852 million by the year 2050 (IDF, 2025). Over 84 million individuals in the Middle East and North Africa Region (MENA) currently live with diabetes, accounting for approximately 17.6% of the adult population. In Algeria, the age-adjusted prevalence of diabetes among individuals aged 20 to 79 years was 17.5% in 2024, representing approximately 4.8 million adults living with diabetes (IDF,

2025). Accumulating evidence has shown that oxidative stress serves as a significant trigger factor promoting the onset of several metabolic diseases, particularly DM and related complications (Chaudhary *et al.*, 2023). Oxidative stress occurs due to higher reactive oxygen species (ROS) production, decreased endogenous antioxidant activity, or both. This imbalance may reduce the release of insulin from pancreatic beta cells and exacerbate insulin resistance in peripheral target tissues (Zhang *et al.*, 2020). Hyperglycemia, a hallmark of diabetes, triggers ROS overproduction, worsening oxidative stress, and low-grade inflammation in adipose tissue (Hurrle and Hsu, 2017). Consequently, several irreversible emerging complications can be the onset of diabetes-related morbidity and mortality (Tomic *et al.*, 2022).

Several anti-diabetic drugs are available to manage diabetes or delay its complications (Dowarah and Singh, 2020). Nevertheless, most of them have many adverse side effects, including gastrointestinal disorders (nausea and vomiting), renal failure, body weight gain, and hypoglycemia (Chaudhury *et al.*, 2017). But, there has been an increasing interest in polyherbal formulations (PHFs) for diabetes prevention and treatment due to their low side effects and toxicity (Suvarna *et al.*, 2021). Moreover, plants are cost-effective and readily accessible (Anwar *et al.*, 2022). PHF has been utilized in traditional medicine systems worldwide for thousands of years and is now gaining popularity in modern medicine due to its high efficacy in a wide range of diseases (Suvarna *et al.*, 2021). Classically, a PHF combines multiple herbs used for therapeutic purposes (Palla *et al.*, 2021). Furthermore, PHFs demonstrated better therapeutic effects than a single herb owing to synergistic pharmacological effects between the various active phytochemical compounds, resulting in significant potential for the treatment of diseases (Perumal *et al.*, 2022).

Numerous clinical and *in vivo* investigations are ongoing to assess the possible benefits of novel polyherbal formulations in the management of DM (Perumal *et al.*, 2022; Khattak *et al.*, 2024; Tanisha *et al.*, 2024). Accordingly, this study sheds light on a twelve medicinal plants-made PHF used in folk medicine in the Adrar region, Algeria, to treat diabetes. We assessed the antioxidant activity of the polyphenol-rich infusion from this PHF. The anti-diabetic effects of polyherbal infusions and polyherbal-based diets on a streptozotocin rat model of diabetes mellitus were also investigated.

MATERIALS AND METHODS

Study location and period: This study was conducted from May 2022 to 2023. Plant materials were collected in May 2022, and all experimental investigations were performed at the Laboratory of Saharan Natural Resources, Faculty of Natural and Life Sciences, University of Adrar, Algeria.

Samples collection and preparation of polyherbal formulation: We made a twelve-herb-based polyherbal formulation (Table 1), belonging to different plant origins. *Nigella sativa* seeds, *Ocimum basilicum* aerial parts, *Coriandrum sativum* seeds, *Lawsonia inermis* leaves, *Sorghum bicolor* seeds, and *Pennisetum glaucum* seeds were harvested in the Adrar region (Southwestern Algeria) from May to October 2022. *Artemisia herba-alba* Asso aerial parts, *Pimpinella anisum* seeds, *Ajuga iva* aerial parts, *Cuminum cyminum* seeds, *Juniperus phoenicea* leaves, and *Ammodaucus leucotrichus* fruits were procured from herbalists.

Before use, all plant materials were meticulously rinsed under running water and then thoroughly air-dried at ambient temperature. Each dried sample was weighed and then powdered using a coffee grinder. We stored each plant's powder at 4 °C in a sterile and airtight container until later experimental assays.

The botanical composition of the PHF and the respective proportions of each plant component are presented in Table 1. The formulation was prepared by homogeneously blending the individually powdered plant materials in specific, non-uniform weights. As our study represents the first pharmacological investigation of this specific PHF advocated in local ethnomedicine for diabetes management, the weights used (Table 1) were determined based on authentic traditional prescriptions provided by local healers and ethnomedicine practitioners of the region. This approach is consistent with established ethnopharmacological methodology in African Traditional Medicine (ATM) and other traditional pharmacopeial systems, in which distinct plant ratios are traditionally employed to achieve synergistic therapeutic effects while minimizing potential toxicity (Hammiche and Maiza, 2006; Evans, 2009; Heinrich *et al.*, 2018).

Preparation of polyherbal extract: The PHF aqueous extract was prepared using the traditional infusion method (Boudjelal *et al.*, 2015). For this purpose, we used 80 mL of boiled distilled water to infuse 4 g of fine PHF powder. The mixture was stirred in a magnetic laboratory agitator for 15 minutes. Afterwards, the extracted solution was centrifuged at 4000 rpm for 20 minutes, followed by separation of the clear supernatant. Evaporation was performed using a rotary evaporator set at 40°C under reduced pressure. Then, the obtained dry extract was subsequently resolubilized in 10 mL of methanol for the subsequent colorimetric assessment of polyphenols and antioxidant capacity. The crude extract was stored in sterile, airtight containers at 4°C until subsequent use. The extraction yield percentage (%) was determined as follows (Ibidapo *et al.*, 2019):

$$Extraction\ yield\ (\%) = \frac{\text{mass of dry extract (g)}}{\text{mass of dried PHF (g)}}\ x\ 100$$

Table 1. Composition of the polyherbal formulation.

Botanical name	Family	Common name	Part used	Weight (g)
Ammodaucus leucotrichus Coss. & Dur.	*Apiaceae*	Saharan cumin	Fruits	10
Coriandrum sativum L.	*Apiaceae*	Coriander	Seeds	20
Cuminum cyminum L.	*Apiaceae*	Cumin	Seeds	35
Pimpinella anisum L.	*Apiaceae*	Anise	Seeds	30
Artemisia herba-alba Asso.	*Asteraceae*	White wormwood	Aerial parts	10
Juniperus phoenicea L.	*Cupressaceae*	Phoenician juniper	Leaves	20
Ajuga iva (L.) Schreb.	*Lamiaceae*	Bugleweed	Aerial parts	20
Ocimum basilicum L.	*Lamiaceae*	Basil	Aerial parts	15
Lawsonia inermis L.	*Lythraceae*	Henna	Leaves	20
Sorghum bicolor L.	*Poaceae*	Sorghum	Seeds	500
Pennisetum glaucum L.	*Poaceae*	Pearl Millet	Seeds	500
Nigella sativa L.	*Ranunculaceae*	Black cumin	Seeds	35

Qualitative Phytochemical analysis: The qualitative analysis of the PHF extract involves a standard series of tests to identify the presence or absence of different classes of bioactive compounds such as flavonoids, phenolic compounds, tannins, alkaloids, cardiac glycosides, reducing sugars, terpenoids, steroids, and saponins, based on the standard procedures (Harborne, 1998; Karumi, 2004; Ayoola *et al.*, 2008).

Quantitative Phytochemical analysis: The quantitative phytochemical analysis involved the determination of total flavonoid content, total phenolic content and condensed tannins content. In the following tests, all tests were performed in triplicate, and the absorbance measurements were taken at 765 nm (TPC), 510 nm (TFC), and 500 nm (CTC) against a reagent blank (methanol for CTC), using a spectrophotometer (Agilent Technologies Cary 60 UV-Vis, USA) (Slinkard and Singleton, 1977).

Determination of Total Flavonoid Content (TFC): TFC was determined following the aluminum chloride ($AlCl_3$) procedure, as previously described (Sakanaka *et al.*, 2005). Briefly, 1250 µL of distilled water was introduced into test tubes containing the PHF crude extract (250 µL), followed by the addition of a $NaNO_2$ solution (75 µL, 5%). Subsequently, 150 µL of aluminum chloride hexahydrate ($AlCl_3 \bullet 6H_2O$, 10%) was introduced after 6 minutes of standing at room temperature, and the mixture was allowed to stand again for 6 minutes under the same conditions. Thereafter, the resulting mixture was mixed with 2 mL of NaOH solution (4%), followed by adjustment of the final volume to 5 mL with distilled water. Finally, all tubes were vigorously shaken and kept at room temperature for 15 minutes. TFC was calculated from the linear regression equation of the catechin standard curve prepared using the same procedure, and the results were reported as milligrams of catechin equivalent per 100 grams of dry weight (mg CE/g DW).

Determination of Total Phenolic Content (TPC): TPC was quantified according to the Folin-Ciocalteu procedure with slight modifications (Slinkard and Singleton, 1977). Briefly, in test tubes comprising 500 µL of the PHF aqueous extract, 2.5 mL of Folin-Ciocalteu reagent and 2 mL of 7.5% sodium carbonate solution were introduced. After that, all tubes were vigorously shaken and maintained in the dark at room temperature for 60 minutes. TPC was quantified from the standard calibration curve of gallic acid, and the results were reported as milligrams of gallic acid equivalent per 100 grams of dry weight (mg GAE/g DW).

Determination of Condensed Tannins Content (CTC): The quantification of CTC was performed using the vanillin method, as previously described (Salar and Purewal, 2017). The reaction mixtures were prepared as follows: 200 µL of the PHF extract was combined with 1500 µL of a methanol-vanillin solution (4%) and 750 µL of hydrochloric acid (37%). The resulting mixtures were allowed to stand at room temperature for 20 minutes of incubation. Then, the CTC was determined from the standard calibration curve of catechin, and the results were presented as milligrams of catechin equivalent per 100 grams of dry weight (mg CE /g DW).

***In-vitro* antioxidant activity:** The antioxidant activities of the aqueous extract of the polyherbal mixture were estimated using three methods: DPPH antiradical activity, ABTS radical inhibitory capacity, and the FRAP test. All tests were performed in triplicates.

DPPH free radical scavenging capacity: The DPPH radical inhibitory action of the PHF aqueous extract was assessed using a standard procedure previously described (Bahiani *et al.*, 2024). The DPPH radical inhibitory action of the test sample, as

well as the standard reference antioxidants (ascorbic and gallic acids), was quantified as the % inhibition of the DPPH, which was calculated as follows:

$$\%\ Inhibition = \frac{A0 - A1}{A0}\ x\ 100$$

Where A_0 represents the absorbance of the DPPH solution, and A_1 is the absorbance of the standard references or test sample.

The antiradical capacities were presented as an IC_{50} (mg/mL) value, which denotes the sample concentration (mg/mL) required to scavenge 50% of the DPPH radical. Additionally, the reaction kinetics of the antioxidant activities were evaluated over 30 minutes, corresponding to the IC_{50} value.

Ferric reducing antioxidant power (FRAP) assay: The reducing ability of the PHF extract was evaluated following a procedure previously reported (Benzie and Strain, 1999). Three milliliters of FRAP solution was mixed with 40 µL of Trolox standard or PHF extract. Following a 30-minute standing at 37 °C, the absorbance measurements were taken at 593 nm. The reducing capacity was determined from the standard calibration curve of Trolox (100–1000 µM /mL), and the results were reported as µmol Trolox equivalent per 100 g dry weight (µmol TE/100 g DW).

ABTS radical scavenging activity: The ABTS radical scavenging activity was assessed using a standard procedure (Re *et al.*, 1999). An amount of 3.9 mL of the freshly prepared ABTS solution was mixed with 100 µL of PHF extract or Trolox standard, followed by incubation for 6 min at room temperature. Afterward, the absorbance measurements were taken at 734 nm, and the scavenging ability was determined from the standard calibration curve of Trolox (100–1000 µM /mL). The findings were reported as micromoles of Trolox equivalent per g of dry weight (µmol TE/g DW).

***In vivo* anti-diabetic activity**

Animals: Adult male Wistar rats (200 - 230 g) were purchased from PIA (Pasteur Institute of Algeria). The animals were then housed in plastic cages and maintained under typical environmental conditions of temperature (22 ± 2°C), 40% relative humidity, and a routine light/dark cycle of 10 h/14 h. Rats were fed a standard diet with free access to water. All experimental procedures involving animals in this study were conducted following the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2011), and complied with the animal housing and welfare standards outlined in the *ARRIVE guidelines 2.0* (Percie du Sert *et al.*, 2020). The research protocol was approved (AP2022/12) by the Research Ethics Committee of Adrar University (Algeria).

Induction of experimental diabetes mellitus: After an acclimatization period of 1 week, diabetes was developed in overnight fasted rats by single intraperitoneal (i/p) injections of 60 mg/kg of STZ freshly dissolved in a 0.1 M sodium citrate buffer (pH 4.5) solution. Moreover, glucose solution (20%) was orally administered to the groups with STZ in order to avoid STZ-induced hypoglycemic mortality (Furman, 2021). Additionally, the control group was intraperitoneally injected with citrate buffer. Fasting blood glucose (FBG) levels were determined 72 hours post-STZ injection via the tail prick method utilizing a Blood Glucose Monitoring Kit (BIONIME, Rightest® GM550, Germany), and the animals with FBG levels ≥ 250 mg/dL were classified as diabetic and selected for the study (Radenković *et al.*, 2016).

Selection of doses: For assessment of the antidiabetic activity in experimental animals, doses of PHF-based diets (*i.e*., PHFD, 25% and 50%) and infusion extracts (*i.e*., PHFI, 0.5% and 1%) were selected based on previous pharmacological studies employing plant-based dietary and infusion regimens in rodent models (Nani *et al.*, 2011; Aboura *et al.*, 2017; Olawole *et al.*, 2018; Alzahrani *et al.*, 2022). For both doses, two distinct concentration levels, moderate and higher doses, were chosen to enable evaluation of the antidiabetic efficacy of PHF in a dose-dependent manner**.**

Experimental design: Thirty male Wistar rats were randomly divided into six groups with five animals (n = 5) using a completely randomized design (CRD), and given the following treatments orally for 4 weeks:
Group 1: Normal Control (NC): Rats received a standard diet and tap water.
Group 2: STZ-Diabetic Control (DC): Rats received a standard diet and tap water.
Group 3: STZ-Diabetic rats treated with 0.5% PHF infusion (PHFI, 0.5%).
Group 4: STZ-Diabetic rats treated with 1% PHF infusion (PHFI, 1%).
Group 5: STZ-Diabetic rats received a diet combined with 25% of PHF (PHFD, 25%).
Group 6: STZ-Diabetic rats received a diet combined with 50% PHF (PHFD, 50%).
The rats' fasting blood glucose levels and body weight were tracked throughout the experiment on days 0, 7, 14, 21, and 28, over four consecutive weeks.

Blood and organs sampling: Following the 28-day experimental period and after overnight fasting, all rats were anesthetized with a single intraperitoneal injection of 40 mg/kg of Zoletil (zolazepam + tiletamine, 1:1), freshly dissolved in phosphate-buffered saline (PBS). Blood samples were directly collected in plain tubes by cardiac puncture. Afterwards, the collected

blood was centrifuged for 15 minutes at 3000 rpm to obtain serum for further biochemical analysis. Also, body organs (kidneys, pancreas, liver, and heart) were collected and weighed.

Biochemical parameters: Different biochemical markers, including uric acid, creatinine, urea, triglyceride (TG), high-density lipoprotein (HDL), total cholesterol (TC), low-density lipoprotein (LDL), total protein (TP), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were estimated in serum using commercially available test kits. In addition, serum corticosterone was analyzed using a commercial ELISA kit (Enzo Life Sciences, Catalog No. ADI-900-097) according to the manufacturer's standardized protocol.

Molecular docking: The molecular docking study was conducted to investigate the plausible mechanism by which PHF reduces hyperglycemia. Briefly, crystallographic structures of four selected diabetes-related proteins were chosen as targets: 5'-adenosine monophosphate protein kinase (AMPK, PDB ID: 6B1U), liver kinase B1 (LKB1, PDB ID: 2WTK), carbohydrate response element-binding protein (ChREBP, PDB ID: 8BTQ), and TBC1 domain family member 1 (TBC1D1, PDB ID: 3QYE). These structures were retrieved from the Protein Data Bank (https://www.rcsb.org/) as PDB files. Following retrieval, water molecules and other heteroatoms were removed from each structure to optimize and stabilize the proteins. Subsequently, polar hydrogen atoms and Kollman charges were added. The prepared proteins were then docked using AutoDock Tools 1.5.7 (ADT) (Morris *et al*., 2009).

p-Coumaric acid (*p*-CA, 3-[4-hydroxyphenyl]-2-propenoic acid), a bioactive compound (PubChem CID: 11790) was used as the ligand for molecular docking study. While metformin, a standard antidiabetic drug (PubChem CID: 4091), served as the comparative ligand. The 3D structures of both ligands were downloaded from the PubChem database (http://PubChem.ncbi.nlm.nih.gov) in SDF format and converted to PDB format using OpenBabel v3.1.1. Hydrogen atoms were added, Gasteiger charges assigned, and root and rotatable bonds detected using ADT. The ligands were then saved in PDBQT format. Grid box coordinates were generated in Discovery Studio, referencing those of the co-crystallized ligand.

Molecular docking was conducted using AutoDock Vina v1.1.2 (Trott and Olson, 2010). Docked poses were ranked by binding affinity (kcal/mol), and the pose with the lowest binding energy was chosen for further study. BIOVIA Discovery Studio Visualizer 2021 was used to visually assess the molecular interactions of the top-docked poses within the binding sites of the selected proteins. Binding energies and interactions were compared to those of metformin.

Statistical analysis: In our study, statistical data analyses were done using GraphPad Prism software 8.0.1 (GraphPad Software, Inc., USA). ANOVA-test (One-way analysis of variance) followed by Tukey's post-hoc multiple comparison test was conducted for comparison between the groups, with $P < 0.05$ regarded as statistically significant. Experimental results were reported as means ± SEM.

RESULTS

Phytochemical analysis: Based on the initial dried plant material, the PHF aqueous extract yield 25% (w/w). Preliminary phytochemical analysis detected the existence of several components, including flavonoids, polyphenols, tannins, steroids, terpenoids, and saponins. In contrast, alkaloids and cardiac glycosides were not detected in the PHF.

Condensed tannins, total flavonoids, and total phenolic contents (CTC, TFC, and TPC, respectively) in PHF extract were assessed using the calibration curve of catechin (R^2 = 0.994), catechin (R^2 = 0.997), and gallic acid (R^2 = 0.996), respectively. Results showed an interesting TPC in PHF, estimated at 1.43 ± 0.03 mg GAE/g DW. More than 60% of the assessed TPC comprises flavonoids, with an amount of 0.82 ± 0.08 mg CE/g DW. CTC was found to be 0.87 ± 0.04 mg CE/ g DW.

In vitro antioxidant activity

DPPH scavenging capacity: Figure 1A displays the DPPH free radical scavenging capacity (% inhibition) of the PHF extract. Our results revealed that the PHF aqueous extract exerted a high DPPH scavenging activity, as indicated by an IC50 of 0.025 ± 0.002 mg/mL. This value was higher than that of ascorbic acid (0.072 ± 0.002 mg/mL) and comparable to that of gallic acid (0.021 ± 0.003 mg/mL).

DPPH scavenging kinetics: The reaction kinetics of DPPH inhibition were conducted to assess the time-dependent behavior of scavenging activity of the PHF extract toward the free radical. Figure 1B illustrates the evolution of the DPPH inhibition by PHF extract compared to standard antioxidants (ascorbic and gallic acids) during a 20-minute incubation period. The results demonstrated that gallic acid exhibited extremely rapid antiradical kinetics; thus, the reaction achieved a steady state within 5 minutes. PHF extract exhibited an intermediate kinetic behavior similar to that of ascorbic acid within the first ten minutes. PHF extract appeared to be more efficient than ascorbic acid over the remaining time.

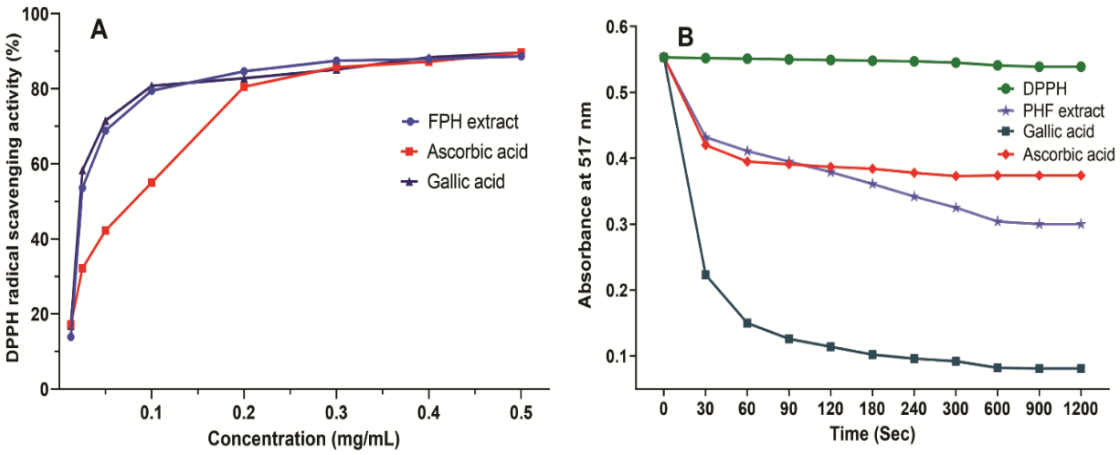

Fig 1. DPPH radical inhibitory activity (A) and the reaction kinetics (B) of the PHF extract and standard reference antioxidants. Values are means ± SEM of three replicate tests. PHF, polyherbal formulation; SEM, standard error of the mean.

Ferric reducing-antioxidant power (FRAP): The reducing ability analyzes the potential ability of a natural antioxidant to convert the ferric ions to ferrous ions by transferring a singlet electron. The reducing capacity of the PHF extract was determined using the calibration curve for Trolox (R^2 = 0.997). Our results demonstrate that the PHF extract displayed a reducing antioxidant power of 3.914 ± 0.115 µmoles TE/100 g DW (Table 2). These findings revealed that the PHF extract exhibits a strong capacity for reducing iron ions.

ABTS radical scavenging activity: The ABTS assay evaluates an antioxidant's ability to neutralize the ABTS free radical by transferring electrons or protons. Table 2 demonstrates that the PHF extract displayed an antioxidant capacity of 4.749 ± 0.069 µmol TE/100 g DW.

Table 2. Antioxidant activities of the PHF as determined via IC_{50}^{DPPH}, FRAP, and ABTS tests.

Samples		IC_{50}^{DPPH} (mg/mL)	FRAP (µmol TE/ g DW)	ABTS (µmol TE/ g DW)
PHF Aqueous extract		0.025 ± 0.002	3.914 ± 0.115	4.749 ± 0.069
References	Ascorbic acid	0.072 ± 0.002	-	-
	Gallic acid	0.021 ± 0.003	-	-

Data are expressed as means ± SEM of three replicate tests. PHF, polyherbal formulation; SEM, standard error of the mean.

In vivo antidiabetic activity

Effect of PHF on fasting blood glucose levels: After 72 hours of diabetes induction, rats had FBG > 250 mg/dL associated with polydipsia, polyphagia, and polyuria. Table 3 displays the FBG levels of normal control, diabetic control, and other experimental groups that received treatment for 28 days. Throughout the experimental period (4 weeks), the diabetic control group exhibited a significant elevation ($P < 0.05$) in FBG compared to the normal control group. Meanwhile, a substantial decrease in the level of FBG ($P < 0.05$) was noticed on days 7, 14, and 21 following the administration of a PHF diet at 25% and 50% to diabetic rats in groups 5 and 6, respectively, resulting in normalized FBG by day 28 compared to the diabetic control group. However, in diabetic rats treated with 1% PHF infusion, the significant reduction in FBG ($P < 0.05$) was not noticed until the 21st day. Conversely, diabetic rats given 0.5% PHF infusion showed a non-significant decrease in FBG levels throughout the study ($P > 0.05$). These results indicate that the PHF extract has a dose-dependent response, with higher concentrations being more effective in lowering FBG levels in diabetic rats. The PHF diets appeared to be the most effective in lowering blood glucose levels compared to the PHF infusions. This result is evidenced by a 78.7% ($P < 0.001$) reduction in FBG levels on the 28th day compared to the initial FBG levels before treatment initiation (Table 3).

Table 3. Effect of PHF on fasting blood glucose levels (mg/dL).

Group (n = 5)	Fasting blood glucose level (mg/dL)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Normal control (NC)	85.60 ± 2.25^{b}	115.20 ± 4.21^{c}	106.40 ± 1.33^{b}	101.40 ± 3.46^{c}	77.00 ± 2.19^{c}
Diabetic control (DC)	362.60 ± 11.51^{a}	365.60 ± 8.04^{a}	370.60 ± 8.57^{a}	375.00 ± 27.63^{a}	383.00 ± 24.18^{a}
Diabetic+PHFI, 0,5 %	371.20 ± 8.74^{a}	357.40 ± 8.39^{a}	349.00 ± 7.14^{a}	332.80 ± 8.05^{a}	308.20 ± 1.85^{a}
Diabetic+PHFI, 1 %	375.40 ± 12.32^{a}	338.80 ± 27.15^{a}	305.40 ± 38.16^{a}	245.80 ± 34.25^{b}	208.80 ± 41.46^{b}
Diabetic+PHFD, 25 %	361.60 ± 11.86^{a}	150.20 ± 5.66^{bc}	138.20 ± 7.51^{b}	115.20 ± 3.84^{c}	81.60 ± 1.60^{c}
Diabetic+PHFD, 50 %	336.00 ± 14.45^{a}	199.80 ± 10.18^{b}	135.40 ± 7.37^{b}	125.60 ± 4.33^{c}	70.80 ± 2.99^{c}

Values are expressed as means ± SEM (n = 5 rats per group). Different superscript letters within the same column indicate statistically significant differences between groups ($P < 0.05$, one-way ANOVA followed by post-hoc test). PHFD, polyherbal formulation diet; PHFI, polyherbal formulation infusion; SEM, standard error of the mean.

Effect of PHF on serum corticosterone levels: As illustrated in Figure 2, the diabetic control group exhibited a significant increment ($P < 0.05$) in corticosterone levels compared to the normal control group. Interestingly, except for the diabetic group receiving a 0.5% PHF infusion (PHFI, 0.5%), all other groups exhibited a marked reduction in serum corticosterone ($P < 0.05$) as compared to the diabetic control group.

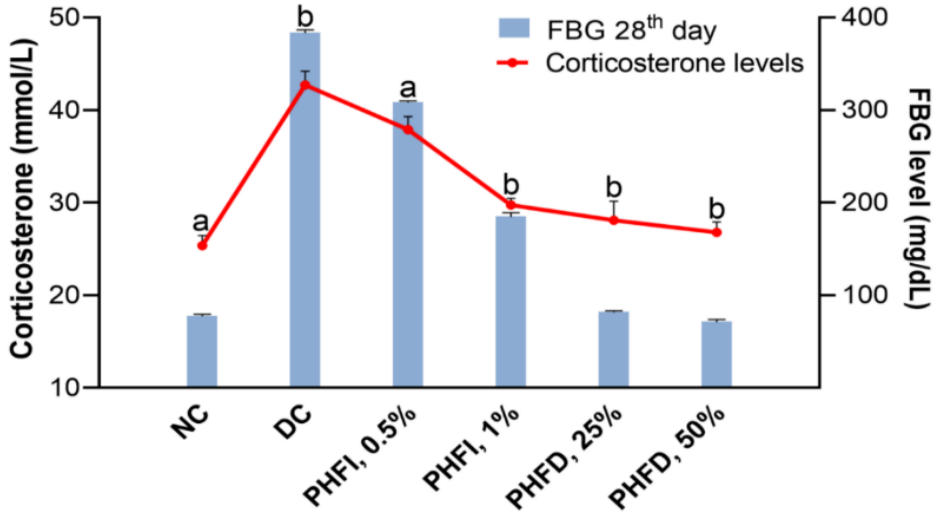

Fig 2. Fasting blood glucose and serum corticosterone levels on the 28th day. Values are means ± SEM (n = 5). Bars marked with different superscript letters exhibit significant differences ($P < 0.05$). SEM, standard error of the mean; DC, diabetic control; NC, normal control; PHFD, polyherbal formulation diet; PHFI, polyherbal formulation infusion.

Effect of PHF on body weight: As shown in Figure 3 (A), there was a marked reduction ($P < 0.05$) in body weight in diabetic rats as compared to the normal control group on the 7th day following the induction of diabetes (STZ). In addition, the diabetic control group showed a statistically significant ($P < 0.05$) decrease in body weight during the experiment compared to their initial body weight, with a weight loss of 10% by the 28th day. Nevertheless, administration of PHFI (1%), PHFD (25%), and PHFD (50%) to diabetic rats resulted in a marked increase in body weight as compared to the diabetic control group, with weight gains of 4.48%, 6.22%, and 8.46%, respectively. However, administration of PHFI with 0.5% to diabetic rats resulted in a slight rise in their body weight, with a weight gain of 0.59% by day 28 (Fig. 3B). The body weight rise in the groups receiving PHF diets was statistically more significant ($P < 0.01$) than in the groups receiving PHF infusions.

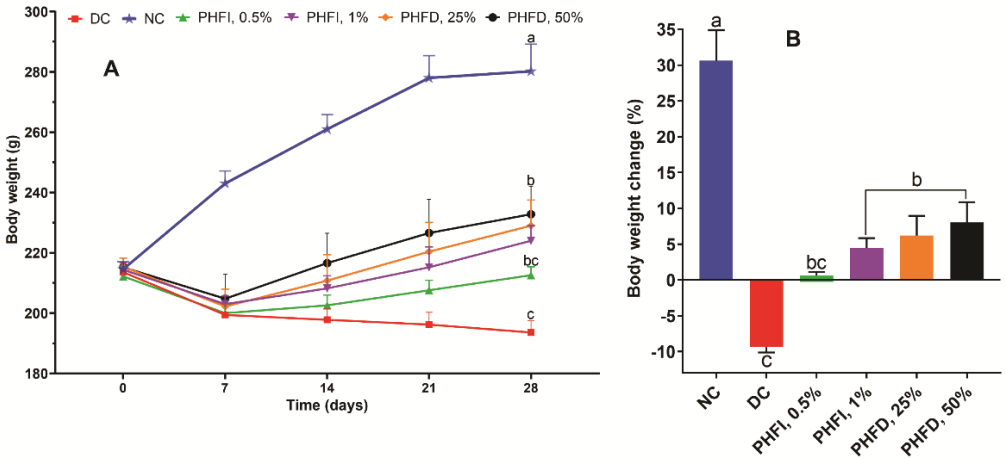

Fig 3. (A) Weekly records of body weight (g) and (B) weight change (%) of control and diabetic rats treated with PHF. Values are means ± SEM (n = 5). Curves with different superscript letters on the 28th day exhibit significant differences ($P < 0.05$). SEM, standard error of the mean; DC, diabetic control; NC, normal control; PHFD, polyherbal formulation diet; PHFI, polyherbal formulation infusion.

Effect of PHF on the relative pancreas, kidney, heart, and liver weights: Relative organ weights (ROW) were determined by collecting and weighing the pancreas, kidney, liver, and heart following 28 days of treatment. As illustrated in Figure 4, there were no statistically significant ($P > 0.05$) changes in the relative weight of the pancreas (RPW) and heart (RHW) across all experimental groups. However, the diabetic control group exhibited statistically significant ($P < 0.05$) increases in relative kidney and liver weights (RKW and RLW, respectively) compared to the normal control group. On the other hand, the administration of PHFI (1%) or PHFDs (25% and 50%) significantly decreased ($P < 0.05$) the RLW and RKW, with the most substantial effect ($P < 0.01$) exhibited by PHFDs.

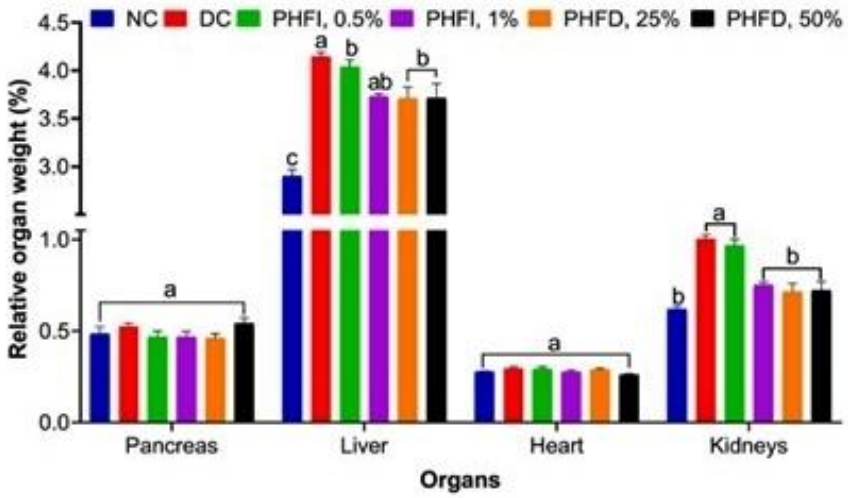

Fig 4. Relative organ weights of normal and diabetic rats. Values are means ± SEM (n = 5). Bars with different superscript letters for each organ exhibit significant differences ($P < 0.05$). SEM, standard error of the mean; DC, diabetic control; NC, normal control; PHFD, polyherbal formulation diet; PHFI, polyherbal formulation infusion.

Effect of PHF on serum lipid profile: As displayed in Figure 5, the diabetic control group exhibited a statistically significant increase ($P < 0.05$) in triglycerides (TG), low-density lipoproteins (LDL), and total cholesterol (TC) levels concomitant with a substantial reduction ($P < 0.05$) in high-density lipoproteins (HDL) concentrations versus normal control group. Meanwhile, diabetic animals that received PHFD (25%), PHFD (50%), and PHFI (1%) exhibited a significant decrease in LDL, TG, and TC levels ($P < 0.05$), accompanied by a considerable increase in HDL levels, compared to the diabetic control group. Interestingly, PHFDs (25% and 50%) have demonstrated the most significant ($P < 0.01$) improvements in lipid profiles as compared to the PHFI (1%).

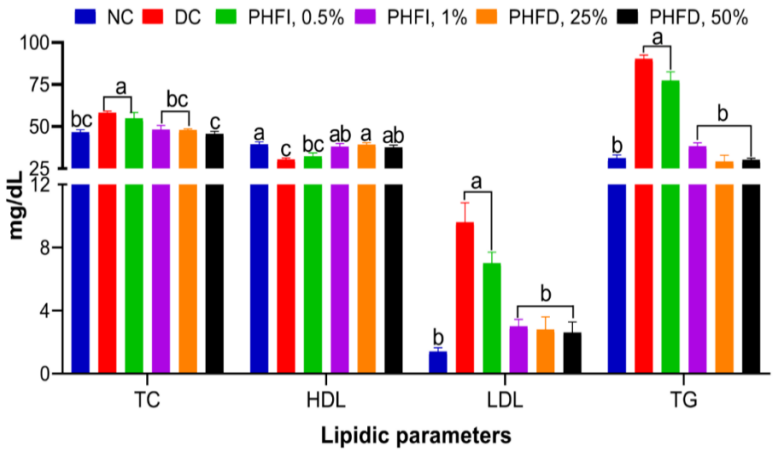

Fig 5. Effect of PHF on lipid profile of control and diabetic rats treated with PHF. Values are means ± SEM (n = 5). Bars with different superscript letters for each organ exhibit significant differences ($P < 0.05$). SEM, standard error of the mean; DC, diabetic control; NC, normal control; PHFD, polyherbal formulation diet; PHFI, polyherbal formulation infusion; HDL, high-density lipoprotein; TC, total cholesterol; TG, triglycerides; LDL, low-density lipoprotein.

Effect of PHF on hepatic biomarker enzymes and total protein: Table 4 displays the serum hepatic biomarkers, namely aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), as well as total protein levels in both control and treated animals. The results indicated that STZ-induced diabetes caused a marked ($P < 0.05$) elevation in serum levels of ALP, ALT, and AST enzymes while also lowering the TP levels. Moreover, diabetic animals that were treated for 28 days with PHF extract (1%), PHF diet (25%), and PHF diet (50%) showed significant ($P < 0.05$) reductions in ALP, ALT, and AST levels compared to the diabetic control group. Meanwhile, there was a marked increase ($P < 0.05$) in TP levels in the diabetic groups receiving PHF infusion (1%) and PHF diets (25% and 50%) compared to the diabetic control group. On the other hand, diabetic rats that received a 0.5% PHF infusion did not exhibit any significant improvement in liver enzyme activities or total protein levels. Interestingly, compared to the PHF infusions, the PHF diets appear to be most effective ($P < 0.01$) in improving total protein and hepatic biomarker enzyme levels.

Table 4. Effect of PHF on some hepatic biomarkers and total protein in treated diabetic and control rats.

Group (n = 5)	Serum liver marker enzymes (UI/L)			Total protein (mg/dL)
	AST	ALT	ALP	TP
Normal control (NC)	142.80 ± 2.92^{cd}	36.40 ± 3.95^{c}	136.60 ± 4.58^{e}	61.40 ± 1.36^{ab}
Diabetic control (DC)	187.40 ± 3.76^{a}	66.60 ± 6.27^{a}	650.20 ± 12.11^{a}	45.20 ± 1.16^{c}
Diabetic+PHFI, 0,5 %	169.80 ± 3.28^{ab}	63.00 ± 2.72^{ab}	625.00 ± 13.10^{ab}	48.60 ± 1.75^{c}
Diabetic+PHFI, 1 %	159.40 ± 5.16^{bc}	50.20 ± 1.66^{bc}	605.20 ± 11.23^{b}	57.00 ± 0.70^{b}
Diabetic+PHFD, 25 %	146.60 ± 6.98^{cd}	47.60 ± 3.88^{bc}	367.20 ± 6.62^{c}	59.40 ± 0.93^{ab}
Diabetic+PHFD, 50 %	131.80 ± 4.68^{d}	44.40 ± 1.08^{c}	303.20 ± 13.51^{d}	62.20 ± 0.86^{a}

Values are expressed as means ± SEM (n = 5 rats per group). Different superscript letters within the same column indicate statistically significant differences between groups ($P < 0.05$, one-way ANOVA followed by post-hoc test). PHFD, polyherbal formulation diet; PHFI, polyherbal formulation infusion; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; SEM, standard error of the mean.

Effect of PHF on kidney function biomarkers: Table 5 displays the serum values of kidney biomarkers, namely creatinine, uric acid, and urea, across all experimental groups. The diabetic control group exhibited a marked ($P < 0.05$) elevation in the assessed serum kidney biomarker levels compared to the normal control group. Moreover, except for the PHF infusion (0.5%) group, significant improvements ($P < 0.05$) in serum kidney biomarker levels were observed in all other PHF-treated groups, with PHF diets appearing to be more effective ($P < 0.01$) in improving kidney biomarkers than PHF infusion.

Table 5. Effect of polyherbal formulation on some kidney function parameters in normal and STZ-induced diabetic rats, after 28 days of treatment.

Group (n = 5)	Serum kidney biomarker levels (mg/dL)		
	Urea	Creatinine	Uric acid
Normal control (NC)	37.00 ± 1.05^{d}	0.39 ± 0.02^{bc}	0.68 ± 0.04^{c}
Diabetic control (DC)	82.20 ± 0.97^{a}	0.51 ± 0.02^{a}	1.36 ± 0.08^{a}
Diabetic+PHFI, 0,5 %	81.60 ± 1.96^{a}	0.44 ± 0.02^{ab}	1.12 ± 0.09^{ab}
Diabetic+PHFI, 1 %	51.60 ± 0.87^{b}	0.39 ± 0.01^{bc}	0.94 ± 0.04^{bc}
Diabetic+PHFD, 25 %	48.40 ± 2.06^{bc}	0.38 ± 0.01^{bc}	0.92 ± 0.06^{bc}
Diabetic+PHFD, 50 %	44.80 ± 1.20^{c}	0.34 ± 0.01^{c}	0.88 ± 0.06^{bc}

Values are expressed as means ± SEM (n = 5 rats per group). Different superscript letters within the same column indicate statistically significant differences between groups ($P < 0.05$, one-way ANOVA followed by post-hoc test). PHFD, polyherbal formulation diet; PHFI, polyherbal formulation infusion; SEM, standard error of the mean.

Potential interaction of *p*-CA with target proteins: Molecular docking analysis was performed to elucidate molecular interactions and binding affinities between the selected compound and the four target proteins. The results of molecular docking focused on binding affinity values (kcal/mol), with more negative values indicating stronger and more stable ligand-protein interactions. The results of free binding energies and types of interactions that occur of *p*-CA and standard metformin at four targeted protein's active sites are summarized in Table 6. Analysis of the docking results reveals that *p*-CA exhibits superior binding affinities and interactions against target proteins compared to standard metformin.

Table 6. Binding energy values and summary of the protein–ligand interactions at the binding site of the proteins.

Ligand	Protein	Binding energy (kcal/mol)	Molecular interactions		
			Hydrogen bonds	Hydrophobic	Van der Waals
p-Coumaric acid (CID: 637542)	AMPK (6B1U)	-6.6	LYS31, GLU19, LEU18	VAL11, VAL81, ILE 46, VAL113	ASN111, ARG83, ASP88, PHE90, ILE115
	LKB1 (2WTK)	-5.6	CYS73, ARG75, THR185, GLY187, THR186	/	HIS231, ARG74, GLU130, THR189
	ChREBP (8BTQ)	-6.5	GLU182, GLU133, GLN67	TYR134	ILE183, TYR131, TRP159, ARG56, TRP130, SER63
	TBC1D1 (3QYE)	-5.5	HIS859, ALA975	LEU980	PHE856, PRO979, ASP759, TYR760, SER976, PHE978, GLN977,
Metformin (CID: 4091)	AMPK (6B1U)	-4.6	ARG83, VAL81, TYR125	/	ASN48, THR87, PHE127, GLU139, ASP136, ILE153, THR85
	LKB1 (2WTK)	-4.9	HIS231, ARG74, GLN251	/	ASP232, THR185, TYR131, ARG75,
	ChREBP (8BTQ)	-5.1	ASN175, ARG128, ARG56	TRP127	ASN124, LYS49, TYR130
	TBC1D1 (3QYE)	-4.7	ARG958, THR764, GLY798, GLN799	/	GLU762, PRO765, CYS766, GLY800, VAL801, ILE763, GLU1039, ILE1042

The interactions between the ligands and target proteins are illustrated in the 2D and 3D representations displayed in Fig. 6. The significant binding affinity of *p*-CA is further substantiated by the 2D diagrams of docking results, which illustrates that *p*-CA establishes more interactions with the target proteins when compared to metformin (Fig. 7).

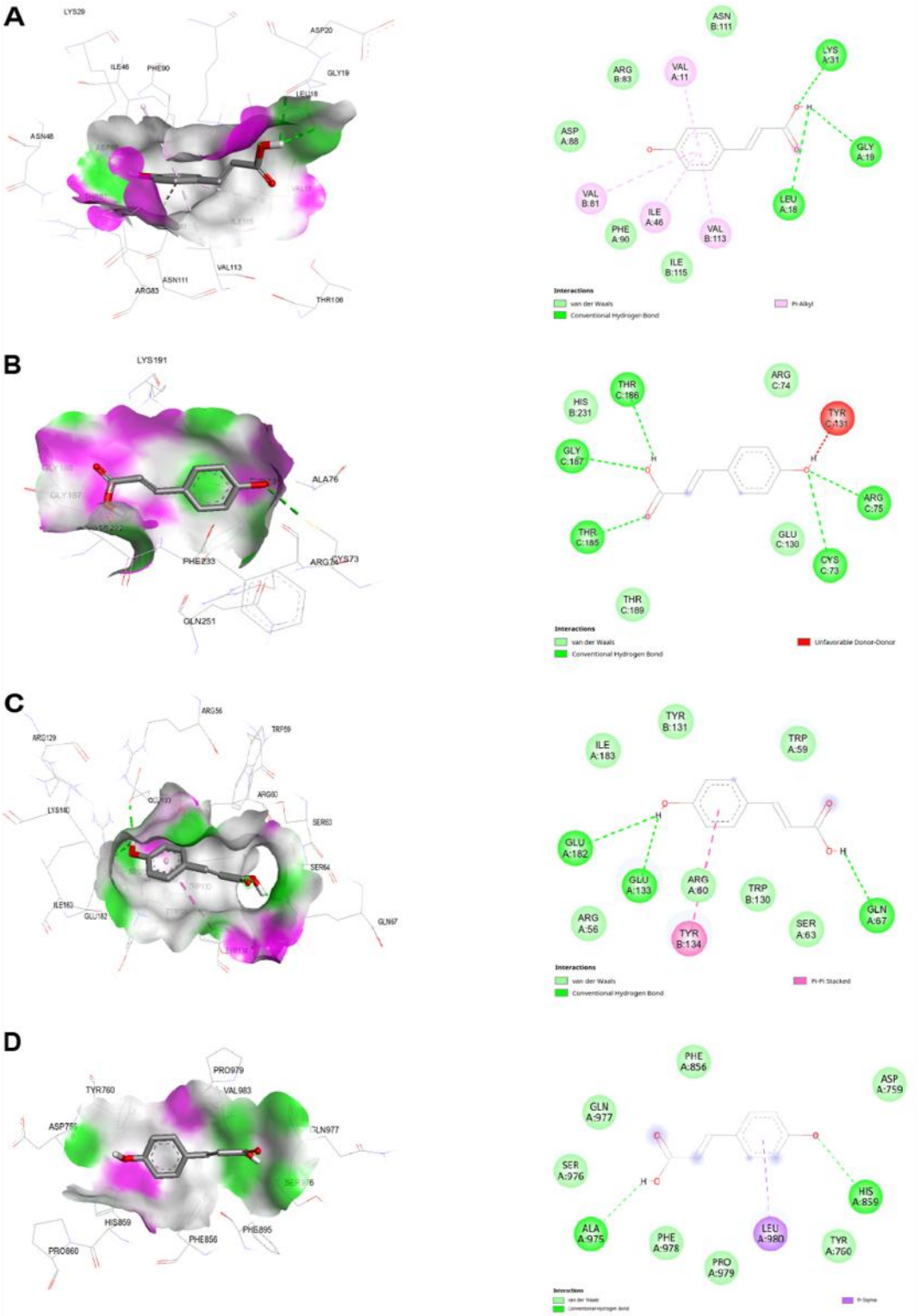

Fig 6. 3D and 2D visualizations of molecular interactions between *p*-CA and target proteins. Each panel displays the 3D structure (left) and 2D interaction diagram (right) for **(A)** AMPK, **(B)** LKB1, **(C)** ChREBP, and **(D)** TBC1D1.

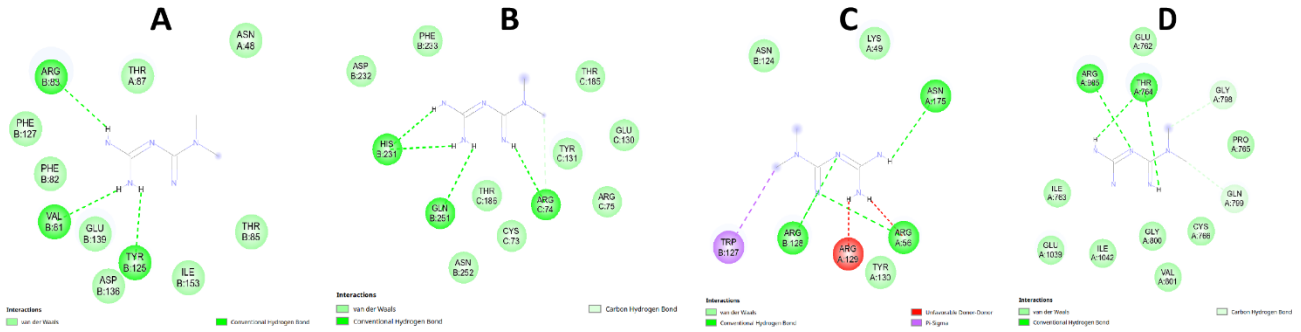

Fig 7. 2D visualization of molecular interactions between metformin and (A) AMPK, (B) LKB1, (C) ChREBP, and (D) TBC1D1 target proteins.

DISCUSSION

Diverse strategies are implemented to manage diabetes, including regular physical exercise and a well-balanced diet (Davies *et al.*, 2022). Moreover, phytotherapy and natural products with anti-diabetic properties are globally regarded as effective alternatives and complementary treatments for managing diabetes (Shahzad *et al.*, 2024). Pharmacological investigations have increasingly focused on the therapeutic efficacy of polyherbal formulations for improving and treating diabetes and its related complications (Khattak *et al.*, 2024; Tanisha *et al.*, 2024). This approach is highly effective thanks to the synergistic effects of multiple herbs and their bioactive components, resulting in greater potency than using a single herb (Perumal *et al.*, 2022; Khattak *et al.*, 2024).

Local populations in the Adrar region (Southwestern Algeria) have historically used a polyherbal formulation (PHF) containing twelve herbs as a supplementary or alternative treatment for managing diabetes mellitus. Hence, the main objective of our study was to analyze and evaluate the effectiveness of this formulation, as well as its potential mechanisms of action in treating hyperglycemia and other metabolic abnormalities related to diabetes in STZ- induced diabetic rats.

Phytochemical screening revealed that the studied PHF mainly contains flavonoids and polyphenols. Indeed, polyphenols were measured at roughly 1.43 ± 0.03 mg GAE/g DW. Previous studies have shown that numerous biological activities of phenolic compounds may be related to their antioxidant capacities (Nani *et al.*, 2021). It has been argued that oxidative stress (OS) represents a significant risk factor for the onset and long-term aggravation of diabetes (Chaudhary *et al.*, 2023). Moreover, reactive oxygen species (ROS) overproduction underlies the primary mechanism by which STZ induces β-cells toxicity in animal models (Szkudelski, 2001). Therefore, restoring the antioxidative/oxidative balance may serve as a promiscuous strategy for diabetes mellitus management. In the present study, the *in vitro* antioxidant ability of the PHF aqueous extract was evaluated using three single-electron transfer (SET)-based methods: DPPH, FRAP, and ABTS assays. PHF aqueous extract exhibited a substantial ability to modulate ROS production and clearance. These findings indicate that the herbs and plants in this PHF are potential sources of antioxidants, which can effectively contribute to maintaining and preserving the functionality of pancreatic β-cells. In this regard, it has been demonstrated that polyphenols exhibit potent antioxidant properties by donating single electrons or hydrogen atoms to reactive free radicals (Chaudhary *et al.*, 2023). Additionally, an *in vivo* study found that administering flavonoids to diabetic animals increases both non-enzymatic and enzymatic antioxidants, resulting in enhanced activity of the antioxidant defense system and thereby conferring protection against pancreatic cell integrity compromised by STZ-induced diabetes (Coskun *et al.*, 2005).

Chronic hyperglycemia, the hallmark of DM, is a major leading cause for the onset of macrovascular and microvascular complications (ElSayed *et al.*, 2023). The present study showed that the diabetic groups experienced a marked increase in FBG on day 0 (FBG ≥ 250 mg/dL) compared with the normal control group, thereby confirming the induction of diabetes (Radenković *et al.*, 2016). Streptozotocin (STZ) is a pancreatic β-cell-targeted cytotoxic agent, typically given in a single high dose, leading to total β-cell death and eventually the onset of diabetes within 48 hours (Furman, 2021). These findings are consistent with previous studies using STZ to induce diabetes (Boudjelal *et al.*, 2015; Choudhari *et al.*, 2017; Bouknana *et al.*, 2024). Importantly, our results indicated that the groups treated with PHF (PHFD, 25%; PHFD, 50%; and PHFI, 1%) for 28 days showed a marked improvement in FBG levels compared with the diabetic control group. However, no significant decrease ($P > 0.05$) was observed in diabetic rats given 0.5% PHF infusion, indicating a dose-dependent antihyperglycemic effect of the PHF. Of all treatments, the PHF-based diet showed a high improvement in FBG levels. Notably, these values fall within the physiological reference range for FBG levels in adult rats (64-200 mg/dL) (Delwatta *et al.*, 2018; Patel *et al.*, 2024). In our study, the decrease in FBG levels noted following PHF treatment may result from the synergistic interactions of PHF phytochemicals. The overall anti-diabetic effects of PHF may be related to its potential to

restore pancreatic tissue function. Previous research demonstrated that an aqueous extract of *Pimpinella anisum* restored pancreatic tissue function in rat models following STZ administration by reducing hyperglycemia-induced oxidative stress (Faried and El-Mehi, 2020). Experimental studies in diabetic rats have shown that methanolic extracts from *Cuminum cyminum* and *Nigella sativa* seeds exert notable antihyperglycemic effects (Jagtap and Patil, 2010; El Rabey *et al.*, 2017).. Accumulating evidence suggests that α-amylase and α-glucosidase are the central enzymes involved in carbohydrate breakdown and digestion, thereby contributing to postprandial hyperglycemia (Gong *et al.*, 2020; Ofosu *et al.*, 2021). Prior research has established that *Pennisetum glaucum* and *Sorghum bicolor*, which are the main constituents of the studied PHF, exhibit high inhibitory efficacy against α-glucosidase and α-amylase enzymes, as well as regulate glucose uptake by peripheral tissues, primarily due to their richness in polyphenols and flavonoids (Ofosu *et al.*, 2021; El Kourchi *et al.*, 2024). Other ingredients in this PHF, such as *Pimpinella anisum* and *Nigella sativa*, have been revealed to enhance insulin secretion from β-cells, as well as promote β-cell proliferation and regeneration by reducing oxidative stress, primarily through their phenolic compounds (Kaatabi *et al.*, 2015; Faried and El-Mehi, 2020). Several single herbs in the studied PHF have demonstrated their antihyperglycemic or hypoglycemic properties. Previous *in vivo* research has shown that administering an aqueous extract from *Ammodaucus leucotrichus* fruits improves hyperglycemia in STZ-induced diabetes (El-ouady and Eddouks, 2019). Single and daily treatment with aqueous extract from *Ajuga iva* and *Artemisia herba-alba* has significantly reduced hyperglycemia in alloxan-induced diabetic rats (Boudjelal *et al.*, 2015). Moreover, an *in vitro* study showed that the aqueous extract from *Ocimum basilicum* leaves exhibited potential hypoglycemic activity by inhibiting α-amylase and α-glucosidase enzymes (El-Beshbishy and Bahashwan, 2011). Another study on *Juniperus phoenicea* revealed that different leaf extracts significantly suppressed the activity of α-amylase (Keskes *et al.*, 2014). Likewise, administration of *Sorghum bicolor* phenolic-rich extract to STZ-treated rats significantly decreased FBG levels by increasing insulin synthesis and secretion, as well as inhibiting hepatic neoglucogenesis (Kim and Park, 2012). Another study showed that administering alloxan-induced diabetic mice with a 70% ethanol extract from *Lawsonia inermis* leaves exhibited hypoglycemic and antihyperlipidemic effects (Abdillah *et al.*, 2008). A study by other researchers demonstrated the hypoglycemic, hypolipidemic, hypocholesterolemia, and hepatoprotective effects of diets containing *Pennisetum glaucum* grains in high-fat diet-fed rats (Alzahrani *et al.*, 2022). *Coriandrum sativum* aqueous extract has also been shown to be effective in decreasing hyperlipidemia and hyperglycemia in obese-hyperglycemic rats (Aissaoui *et al.*, 2011).

Besides the common diabetic signs of polydipsia, polyphagia, and polyuria associated with hyperglycemia, body weight loss is known to be a hallmark of untreated diabetes (Chen *et al.*, 2017). Streptozotocin-induced diabetic models have also been reported to experience progressive body weight loss (Ab Rahman *et al.*, 2020). This body weight loss in rodents could be attributed to insulin deficiency-associated increased fatty acid oxidation and protein catabolism as alternative energy sources (Ab Rahman *et al.*, 2020). Interestingly, diabetic groups receiving PHF diets (25 and 50%) showed significant weight gains compared to the diabetic control rats. These results align with prior studies that validated the protective action of PHFs against muscle wasting (Choudhari *et al.*, 2017; Madić *et al.*, 2021). Furthermore, *Ammodaucus leucotrichus* polyphenols-rich extract prevented weight loss and improved diabetes-related biochemical parameters (El-ouady and Eddouks, 2019; Bouknana *et al.*, 2024).

It is well known that abnormal lipid profiles and lipoproteins are mostly coupled with hyperglycemia and are one of the most common pathophysiological changes in uncontrolled DM, potentiating the leading cause of serious complications, including coronary heart disease (Yun and Ko, 2021). Diabetic dyslipidemia can be attributed to an increase in the mobilization of fatty acids from adipocytes, resulting from the underutilization of glucose due to insulin resistance or deficiency (Thambiah and Lai, 2021). In addition, reduced high-density lipoproteins (HDLs) levels and elevated low-density lipoproteins (LDL), triglycerides (TG), and total cholesterol (TC) levels are the leading indicators of diabetic dyslipidemia (Madić *et al.*, 2021). In our study, the administration of PHF-based diets (*i.e.*, PHFD, 25% and PHFD, 50%) and PHFI (1%) had a significant impact on modulating serum lipid profiles, as evidenced by increases in HDL and decreases in TC, TG, and LDL levels in diabetic rats. Our findings align with prior research indicating that *Ojamin*, a PHF, significantly enhanced the serum lipid profile in diabetic rats (Choudhari *et al.*, 2017). It has been suggested that PHF phytochemical compounds, such as saponin and flavonoid, play a critical role in regulating lipid metabolism, resulting in a normalized serum lipid profile (Zhang *et al.*, 2023). Evidence has shown that *Coriandrum sativum* has effectively improved lipid metabolism in animal models, as revealed by restored serum lipid levels (Kajal and Singh, 2019).

Corticosterone, a rodent glucocorticoid, is commonly associated with glucose and lipid signaling abnormalities in diabetes (Hao *et al.*, 2021). DM is a long-term metabolic stressor that mainly damages the function of the hypothalamic-pituitary-adrenal (HPA) axis, characterized by increased circulating glucocorticoid levels (corticosterone in rodents or cortisol in humans) associated with a decreased glucocorticoid negative feedback sensitivity (Mosili *et al.*, 2024). In line with this, our results revealed that STZ-induced diabetic rats significantly increased serum corticosterone levels, which were associated with elevated fasting blood glucose levels. The elevated corticosterone levels could result from hyperglycemia associated with oxidative stress-induced HPA axis dysregulation (Oyouni *et al.*, 2022). In this context, it has been proposed that increased corticosterone levels in STZ-diabetic rats are due to chronic hyperglycemia and/or hypoinsulinemia (Chan *et*

al., 2001). In the present study, administering the PHF (PHFD, 25 and 50%) for 28 days significantly decreased corticosterone levels compared to diabetic control rats, possibly due to improved insulin secretion and enhanced antioxidant status. A previous study demonstrated that insulin administration to diabetic rats restored normal corticosterone levels (Chan *et al.*, 2002). Furthermore, it has been found that antioxidant treatments effectively inhibited ROS generation and reinstated the adrenal cortex's functional ability (Repetto *et al.*, 2019).

The liver maintains lipid and glucose homeostasis through regulating multiple metabolic processes, such as glycogenesis and lipid synthesis, in response to insulin (Guerra and Gastaldelli, 2020). DM is one of the major leading causes of hepatic injuries, particularly metabolic dysfunction-associated steatotic liver disease (MASLD), which is generally accompanied by overaccumulation of fat in hepatocytes, primarily as triglycerides, in the absence of alcohol consumption (Otero Sanchez *et al.*, 2024). It is well established that in addition to its primary target, pancreatic beta cells, STZ can also induce damage to other organs, including the kidney and liver (Lenzen, 2008). The observed alteration in serum liver function biomarkers, such as alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP), along with the decrease in total protein (TP) level and increase in relative liver weight (RLW) in diabetic control group indicate hepatocellular injury, as previously argued (Ab Rahman *et al.*, 2020). Emerging evidence suggests that the administration of STZ can induce hepatotoxicity damage through ROS overproduction due to high levels of blood glucose and insulin deficiency, ultimately leading to hepatic injury (Arabloei Sani *et al.*, 2022). Indeed, it is well known that ROS can damage proteins and lipids, which are the main components of cell membranes. Consequently, damaged hepatocyte membranes permit the release of liver enzymes into the bloodstream (Mohamed *et al.*, 2016). Nevertheless, the PHF diabetic-treated groups (PHFD, 25 and 50%) showed manifest improvement in liver function biomarkers and a significant decrease in RLW, indicating that this PHF exhibits prominent hepatoprotective properties. The potential hepatoprotective effect of PHF might be attributed to its abundance of phenolic compounds, which serve as antioxidant agents. In addition, numerous studies in the past few years have demonstrated that the hepatoprotective effects of phenolic compounds are closely associated with their antioxidant activities (Darvin *et al.*, 2018; Teofilović *et al.*, 2021; Alzahrani *et al.*, 2022).

Diabetic kidney disease, also known as diabetic nephropathy, is a severe microvascular complication that affects patients with T1DM (Papadopoulou-Marketou *et al.*, 2017). It can arise as a consequence of chronic hyperglycemia-induced oxidative stress, which causes kidney damage by triggering abnormal changes in the kidney cells' structure and function (Samsu, 2021). The increase of renal biomarkers in the blood, including urea, creatinine, and uric acid, is a significant signal of diabetic nephropathy due to impaired kidney capacity to filter these metabolic byproducts (Ikewuchi *et al.*, 2017). We noticed an increase in these biomarkers, accompanied by a substantial increase in relative kidney weight (RKW) in diabetic rats. Conversely, compared to the diabetic control rats, PHF treatments, particularly the PHF-based diet, resulted in a marked decrease in serum renal biomarkers. In line with the results obtained, previous research has found that diabetic rats administered *Coriandrum sativum* seed extract showed a pronounced decrease in serum renal marker levels over four weeks, accompanied by an RKW reduction. The same authors reported that these improvements were due to inhibiting the kidneys' advanced glycation end products (AGEs) generation (Kajal and Singh, 2019). Another study found that treating diabetic rats with a PHF containing *Cuminum cyminum* and *Nigella sativa* significantly improved renal function through its antioxidant and anti-diabetic properties (Anwar *et al.*, 2022). Previous investigations have shown that dietary polyphenols, due to their antioxidant and anti-inflammatory activities, can delay the progression of diabetic nephropathy (Liu *et al.*, 2023; Zhao *et al.*, 2024).

A notable limitation of this study is that histopathological examinations of the liver, kidney, and pancreas were not performed. Consequently, our conclusions regarding the protective effects of PHF are based solely on functional biomarkers and relative organ weights, reflecting biochemical and metabolic improvement rather than structural tissue preservation. Future studies incorporating histopathological analyses are warranted to substantiate these findings at the morphological level.

Molecular docking is an effective computational approach for mechanistically validating traditional polyherbal formulations used in the management of diabetes mellitus, as evidenced by published studies on medicinal formulations in traditional systems (Telapolu *et al.*, 2018; Kumar *et al.*, 2022; Jadhav and Noor, 2025). In this study, docking estimates binding affinities, orientations, and key interactions between *p*-CA and diabetes-related protein targets, thereby offering preliminary insight into its antidiabetic activity and plausible mechanism of action for the studied PHF.

The selection of this bioactive compound was based on previous studies reporting that *p*-CA represents the most dominant phenolic acid in the key ingredients (pearl millet and sorghum) of the PHF (Nani *et al.*, 2015; Mawouma *et al.*, 2022; Triki *et al.*, 2025). Furthermore, *p*-CA has garnered considerable research attention due to its potential pharmacological activities. Prior *in silico*, *in vitro*, and *in vivo* investigations have demonstrated that *p*-CA possesses a wide range of activities pertinent to the pathophysiology under study, including antidiabetic, antioxidant, anti-inflammatory, hepatoprotective, and nephroprotective effects (Pragasam *et al.*, 2013; Yoon *et al.*, 2013; Amalan *et al.*, 2016; Li *et al.*, 2022; Mani *et al.*, 2022; Mehdi *et al.*, 2022; McMillan *et al.*, 2025).

Our molecular docking results suggest that *p*-CA may interact with AMPK, LKB1, ChREBP, and TBC1D1, which are known to mediate metabolic regulation. Notably, *p*-CA acid demonstrated higher predicted affinities for these target proteins than metformin under the simulation conditions. *p*-CA was predicted to interact with protein binding sites via several amino acid residues, demonstrating strong binding affinities. Docking analyses demonstrated that *p*-CA's binding was mediated by hydrophobic, hydrogen bonding interactions, and van der Waals forces, consistent with previous findings revealing that these noncovalent forces cooperatively stabilize ligands at their target sites (Patil *et al.*, 2010).

It is important to highlight that while AMPK is recognized as a crucial metabolic sensor responding to cellular energy balance (Hunie Tesfa *et al.*, 2025), and is pivotal in diabetes management by modulating physiological processes that promote β cell function, insulin sensitivity, and glucose metabolism (Entezari *et al.*, 2022), our data only support the plausibility of *p*-CA interacting with these pathways from a computational perspective. A prior study indicated that *p*-CA increased glucose uptake in skeletal muscle cells and other cells by acting as an AMPK activator, suggesting it may prevent or ameliorate insulin resistance and type 2 diabetes through the modulation of lipid and glucose metabolism (Yoon *et al.*, 2013). Liver kinase B1 (LKB1) functions as a master kinase that directly activates AMPK in muscle and liver cells, thereby regulating lipid and glucose metabolism. A previous research has shown that LKB1-mediated AMPK activation serves as a key regulator of glucose homeostasis by enhancing insulin sensitivity in skeletal muscle (Wang *et al.*, 2019). Carbohydrate response element binding protein (ChREBP), a transcription factor, serves as a glucose-dependent regulator of carbohydrate signaling and enhances fatty acid synthesis and triglyceride accumulation in hepatic cells. Targeting ChREBP presents opportunities for the development of therapies for hepatic steatosis and diabetes mellitus (Régnier *et al.*, 2023; Devnath *et al.*, 2025).TBC1D1 is a member of the TBC1 Rab-GTPase-activating protein (Rab-GAP), predominantly expressed in skeletal muscle, which enhances glucose absorption mainly via regulating GLUT4 translocation (Hook *et al.*, 2020). In the current study, *p*-CA exhibited strong predicted affinities with LKB1, ChREBP, and TBC1D1 with binding energies of -5.6, -6.5, and -5.5 kcal/mol, respectively.

These computational findings offer preliminary evidence supporting the potential use of this polyherbal formulation as an antidiabetic supplement. However, it must be emphasized that activation of AMPK, LKB1, or related signaling pathways was not experimentally verified in this study, and molecular docking alone cannot demonstrate pathway activation *in vivo*. The proposed molecular mechanisms and physiological effects—including improved pancreatic β-cell function, enhanced insulin sensitivity, and increased glucose uptake—remain hypothetical, based on prior literature and the predictive nature of molecular docking. Future experimental validation via gene expression, Western blotting, enzyme activity assays, or immunohistochemistry will be essential to confirm these hypotheses and clarify the actual mechanisms underlying any observed therapeutic benefits.

The key finding of this study is that the PHF-based diet-treated groups exhibited more significant anti-diabetic activity. An essential feature of the studied PHF is its abundance of whole grains, edible seeds, herbs, and spices. These ingredients have been traditionally used in the Mediterranean region for both medicinal and culinary uses (Salas-Salvadó *et al.*, 2016). Additionally, we prepared the PHF according to the traditional procedure in local folk medicine. Interestingly, traditional healers often prescribe this PHF for oral administration in powdered form, known as "*Saffa*" in the Southwestern Algerian Sahara. Therefore, several researchers have demonstrated the long-standing use of this method in folk medicine (Swanston-Flatt *et al.*, 1990; Hameed *et al.*, 2020; Suvarna *et al.*, 2021). Besides the bioactive compounds already screened in this mixture, whole grain cereals and edible seeds feature a lower glycemic index, as well as are abundant with several phytonutrients, including soluble dietary fibers, monounsaturated fatty acids (MUFA), and micronutrients (Pei *et al.*, 2022). It has been reported that whole grains reduce the risk of DM via modulating targeted hepatic glucose metabolism (Wang *et al.*, 2024). A meta-analysis study revealed that sorghum and millets possess significant efficacy in the dietary management of diabetes, primarily due to their low glycemic index (GI), rich fiber, high polyphenol, and antioxidant contents (Anitha *et al.*, 2021). Moreover, increasing evidence indicates a positive correlation between high consumption of whole grains and a decreased risk of DM, which is attributed to modulating cellular glucose uptake and inhibiting the α-amylase and α-glucosidase enzymes (Gong *et al.*, 2020).

Conclusion: The current investigation serves as the preliminary investigation of the phytochemical screening, antioxidant, and antidiabetic activities of a novel PHF. Our results indicate that the PHF exhibited significant antioxidant activities. Additionally, administering the PHF substantially improved body weight gain, glycemia, and lipidemia, as well as the reno-protective and hepatoprotective properties in STZ-induced diabetic rats, thus suggesting its strong anti-diabetic potential. These findings offer valuable insights into the potential application of this PHF in complementary and alternative medicine for diabetes management. However, future research should prioritize the formal dose–response optimization of the plant ratios, followed by the identification of the main phytochemical compounds and the exploration of the synergistic effects of the most dominant ones. Moreover, elucidating the main signaling pathways modulated by PHF may help in understanding its antidiabetic mechanism at the cellular level.

Ethics approval: All experimental procedures involving animals in this study were conducted following the *Guide for the Care and Use of Laboratory Animals* and complied with the animal housing and welfare standards outlined in the *ARRIVE guidelines 2.0*. The research protocol was approved (AP2022/12) by the Research Ethics Committee of Adrar University (Algeria).

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