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Phytochemical profile and antioxidant capacity, α-amylase and α-glucosidase inhibitory activities of *Oxalis pes-caprae* extracts in alloxan-induced diabetic mice

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ABSTRACT

Diabetes and its complications are closely correlated with chronic hyperglycemia, causing severe oxidative stress and leading to glycation reaction with formation of advanced glycation end products. However, medicinal plants are still a source of inspiration for the discovery of new treatments of several diseases, including diabetes. The present study was aimed to evaluate the antioxidant and antidiabetic properties of *Oxalis pes-caprae* flowers extract in alloxan-induced diabetic mice. The phytochemical and antioxidant activities of both aqueous and methanolic extracts were assessed by in-vitro testing such as free radical scavenging assays (DPPH and $ABTS^{+}$), ferrous ions (Fe^{2+}) chelating activity and reducing power assay. Additionally, the detection of Amadori products and advanced glycation end products was used to determine the antiglycation potential. α-glucosidase and α-amylase inhibitory assessment was employed to determine the antidiabetic effect, while alloxan-induced diabetic mice were used to measure the in-vivo activities of antioxidants and carbohydrates enzymes. The effect of the methanolic extract on body weight and blood glucose level of extract-treated diabetic mice were also investigated. Among the tested extract, the methanolic extract was the richest in phenolic compounds which is directly related with their remarkable antioxidant, enzyme inhibitory and antiglycation activity. The oral administration of the two doses of *Oxalis pes-caprae* flowers (150 mg/kg and 250 mg/kg) daily for 3 weeks resulted in hypoglycemic effect compared to the reference drug, glibenclamide (10 mg/kg). Furthermore, the extract was shown to significantly increase the activities of antioxidants and glycolysis enzymes in the liver,

Abbreviations: AAE, Ascorbic acid Equivalent; ABTS, 2,2′-azino-bis(3ethylbenzothiazoline-6-sulfonic acid; AG, Aminoguanidine; AGEs, Advanced glycation end productions; BSA, Bovine serum albumin; CAT, Catalase; DM, Diabetes mellitus; 1-DMF, GPx: glutathione peroxidase; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EDTA, Ethylenediaminetetraacetic acid; HDL, High-density lipoprotein; HK, Hexokinase; LDL, Low-density lipoprotein; FRAP, Ferric reducing antioxidant power; GAE, Gallic Acid Equivalent; G6PD, Glucose-6-phosphate dehydrogenase; GR, glutathione reductase; H_2O_2, Hydrogen peroxide;1-deoxy-1-morpholino-fructose; MDA, Malondialdehyde; OECD, Economic cooperation and development; PEP, Phosphoenolpyruvate; PK, Pyruvate kinase; QE, Quercetin Equivalent; RAGE, AGE-specific receptor; ROS, Reactive oxygen species; SOD, superoxide dismutase; TBA, Thiobarbituric acid; TFC, total flavonoid content; TG, Triglyceride; TPC, total phenolic content.

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kidney and spleen of diabetic mice, compared to diabetic control group. Therefore, *Oxalis pes-caprae* extract effectively exhibited hypoglycemic and antidiabetic effects as indicated by in-vitro and in-vivo studies, confirming the protective effects on hyperglycemia and oxidative damage.

1. Introduction

Diabetes mellitus (DM) remains a global epidemic with severe health and socioeconomic burdens. It is becoming one of the top five leading causes of death in the 21st century. According to the World Health Organization report, the number of diabetic patients is anticipated to rise to 643 million by 2030 globally [1]. Extensive evidence reported that the pathophysiology of diabetes complications is linked to a combination of increased reactive oxygen species (ROS) and alteration of the antioxidant defense systems [2]. The imbalance of redox homeostasis enhances lipid peroxidation, impairment of glutathione metabolism, mitochondrial dysfunction, metabolism deregulation (e.g. Altered levels of serum triglyceride (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) [3,4], etc. Moreover, persistent hyperglycemia induces different structural and functional changes in proteins through a process called glycation and generates high levels of advanced glycation end productions (AGEs) [5,6]. These AGEs are responsible for the development of microvascular complications (nephropathy, retinopathy, and neuropathy) and macrovascular complications (cerebrovascular, cardiovascular and peripheral vascular diseases) [7].

There are several synthetic hypoglycemic drugs for the treatment or management of diabetes, such as metformin and sulfonylureas [8,9]. However, due to their significant adverse effects that outweigh their therapeutic effects, these medications do not provide adequate results [10]. In contrast to this conventional pharmaceuticals, herbal remedies are widely used for diabetes prevention because of their accessibility, relative safety and wide range of biological activity [11,12]. Secondary metabolites of medicinal plants have piqued interest as a potential source of hypoglycemic treatments and a safer alternative to manufactured medications. Indeed, they may affect blood glucose levels through various mechanisms, including inhibition of key hyperglycemia-related enzymes such as α-glucosidase and α-amylase [13]. They may also be potent inhibitors of free radical formation generated by hyperglycemia [14].

Oxalis pes-caprae (*O. pes-caprae*), which belongs to *Oxalidaceae* family is commonly known in the north of Morocco as “hmayda” because of its sourness [15]. It is a perennial herbaceous plant with bulbiferous and subterranean stems that grows between 12 and 24 cm tall [15]. Although it grows like a weed, it is edible and non-toxic in little amounts [16]. It contains luteolin and apigenin derivatives, 3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, and chlorogenic acid [17]. Polyphenolic substances derived from aerial component extracts were discovered to have strong antioxidant activity [18]. Previous studies have also demonstrated that *O. pes-caprae* has an antibacterial and antifungal activities [16,19]. In Morocco, *O. pes-caprae* is widely used in folk medicine to treat diabetes, hypertension, dermatological and circulatory disorders, influenza, bronchitis and pharyngitis [20].

The present study aims to investigate the antioxidant activities of methanolic extract from *O. pes-caprae* flower, antiglycation and antidiabetic properties. To the best of our knowledge, this study is the first to report the in vivo antidiabetic efficacy of the methanolic extract of *O. pes-caprae* flowers against alloxan-induced diabetes mice model.

2. Experimental section

2.1. Plant material

Plant was grown in the suburbs of Tangier, Morocco. The plant was identified by Mohamed El kadiri, botanist at the Faculty of Sciences Tetouan, Morocco. A voucher specimen (No. 1–130835) was deposited at the herbarium of the Faculty of Sciences and Techniques, Tangier (Morocco). The flowers and leaves of *O. pes-caprae* were washed with distilled water, shade dried approximately for 2 weeks at room temperature in relative humidity of the laboratory and powdered to achieve a mean particle size. The powder was kept in the dark until future analysis.

2.2. Plant extraction

The plant powder was extracted by maceration with either distilled water or methanol 80% (45 mL/4.5 g of plant powder) at room temperature for 24 h. Centrifugation at 5000 g for 10 min and filtration with the Whatman filter were conducted. The water and methanol solvents were then dried in an incubator at 40 °C, taking 7 and 3 days, respectively. The dried extracts were stored until use for further analysis.

2.3. HPLC-DAD-ESI/MS analysis

HPLC-DAD-ESI/MS analysis of polyphenolic compounds from the methanolic extract of *O. pes-caprae* was conducted on a Shimadzu liquid chromatography system (Kyoto, Japan) consisting of a CBM-20A controller, two LC-20 CE dual-plunger parallel-flow pumps, a DGU-20A5R degasser, a SIL-20AC autosampler, an SPD-M30A photo diode array detector and an LCMS-8050 mass spectrometer, through an ESI source (Shimadzu, Kyoto, Japan).

Chromatographic separations were performed on 150 × 4.6 mm; 2.7 μm Ascentis Express RP C18 column (Merck Life Science, Merck KGaA, Darmstadt, Germany). The mobile phase was composed of two solvents: water/acetic acid (99.85/0.15 *v/v*, solvent A) and acetonitrile/acetic acid (99.85/0.15 *v/v*, solvent B), The flow rate was fixed at 1 mL/min under gradient elution: 0–5 min, 5% B, 5–15 min, 10% B, 15–30 min, 20% B, 30–60 min, 50% B, 60 min, 100% B. DAD detection was applied in the range of λ = 200–400 nm and a wavelength of 280 nm was monitored (sampling frequency: 40.0 Hz, time constant: 0.08 s). MS conditions were as follows: scan range and scan speed were set at *m/z* 100–900 and 2500 u s^{-1}, respectively, event time: 0.3 s, nebulizing gas (N_2) flow rate: 1.5 L min^{-1}, drying gas (N_2) flow rate: 15 L min^{-1}, interface temperature: 350 °C, heat block temperature: 300 °C, DL (desolvation line) temperature: 300 °C, DL voltage: 1 V, interface voltage: − 4.5 kV.

2.4. Determination of total phenolic content

Total phenolic content of *O. pes-caprae* flower extract was determined using Folin–Ciocalteu method [21]. Briefly, 10 mg of powder extract was dissolved in 10 mL of deionized water. 400 μL of Folin-Ciocalteu reagent (Merck KGaA, Darmstadt, Germany) freshly prepared was added to 100 μL of flower extract; then, to this mixture 1000 μL of 7% sodium carbonate and 100 μL of deionized water were added and allowed to incubation in the dark for 30 min at room temperature. The absorbance of the mixture was measured at 725 nm using spectrophotometer (EPOCH, BioTek). Gallic acid (Merck KGaA, Darmstadt, Germany) was used as a standard control to quantify the total phenolic contents.

2.5. Determination of total flavonoid content

To measure the flavonoid content, we referred to the method of Huang et al. with some slight modifications, as reported by Bouchmaa et al. [22]. Briefly, the mixture containing 40 μL of flower extract, 10 μL

of 1 M acetate potassium and 10 μL of 10% aluminum chloride was homogenized. Thereafter, 100 μL of 50% methanol was added and the total volume was made up to 400 μL with deionized water. After incubation at room temperature for 30 min in the dark, the absorbance of the mixture was taken at 415 nm. Quercetin (Merck KGaA, Darmstadt, Germany) was used as standard control.

2.6. Antioxidant activities

2.6.1. DPPH radical scavenging assay

The radical scavenging ability was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl) and following the method described by Ben Mrid et al. [23] with some modifications. Adequate dilutions of leaves and flowers of *O. pes-caprae* were realized to obtain a final volume of 50 μL. The extract solutions (50 μL) were mixed with 150 μL of a freshly prepared DPPH solution (Sigma-Aldrich, United States). After mixing, the mixture left to stand in the dark and at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm. The DPPH scavenging activity was determined by the following equation:

$$\%\text{Scavenging effect} = [(A_{DPPH} - A_S)/A_{DPPH}] \times 100$$

A_S correspond to the sample absorbance; A_{DPPH} is referring the absorbance of the DPPH solution. The extract concentration providing 50% inhibition (IC_{50}) was determined from the graph of scavenging effect percentage against extract concentration in the solution.

2.6.2. $ABTS^+$ radical scavenging assay

The radical scavenging effect of the extracts obtained from *O. pes-caprae* leaves and flowers was assessed against the radical $ABTS^+$ following the method of Re et al. [24]. $ABTS^+$ was generated by the using of potassium persulfate. Prior to assay, the $ABTS^+$ (Sigma-Aldrich, United States) stock solution was diluted with methanol until its reach an absorbance of 0.700 ± 0.020 at 734 nm. From a diluted solution of $ABTS^+$, 185 μL was mixed with 15 μL of each sample. The absorbance of mixtures was measured at 734 nm after 10 min. The following equation has been used to calculated radical scavenging activity:

$$\%\text{Scavenging effect} = [(A_{ABTS} - A_S)/A_{ABTS}] \times 100$$

A_S correspond to the sample absorbance and A_{ABTS} is referring to the absorbance of the ABTS solution. The extract concentration providing 50% inhibition (IC_{50}) was determined from the graph of scavenging effect percentage against extract concentration in the solution.

2.6.3. Metal chelating activity

The ferrous ion chelating potential for extracts of leaves and flowers was determined by the protocol proposed by Dinis et al. [25]. The reaction mixture is composed of 800 μL of increasing concentrations of the extracts and 10 μL of $FeCl_2$ (0.6 mM). After vigorous mixing, the glass tubes left to stand at room temperature for 10 min. After that, 50 μL of ferrozine (Sigma-Aldrich, United States) (5 mM) was added, and the final volume was made up to 1 mL with distilled water. The absorbance of the reaction mixture was measured after 10 min at 562 nm. The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of scavenging effect percentage against extract concentration in the solution.

2.6.4. Ferric reducing antioxidant power (FRAP) assay

The reducing power of the *O. pes-caprae* leaves and flowers extracts was evaluated using the protocol of Oyaizu [26] with some modifications. In glass tubes, 200 μL of sample was added to 500 μL of phosphate buffer (0.2 M, pH 6.6) and 500 μL of potassium ferricyanide (1%), and the mixture was incubated at 50 °C for 20 min after incubation, 500 μL of trichloroacetic acid (10%) was added and centrifuged for 10 min. Thereafter, 500 μL of supernatants was mixed with 500 μL of distilled water and 100 μL of ferric chloride (0.1%). Absorbance of solutions was measured at 700 nm, and the values are presented as mg of ascorbic acid equivalent per g of dry weight.

2.7. Antiglycation activities

The BSA-fructose model was achieved following to a previously work reported by Mrid et al. [27] to evaluate the inhibitory effect of *O. pes-caprae* on the glycation process. In brief, fructose (Sigma-Aldrich, United States) (0.5 M) dissolved in potassium phosphate buffer (pH 7.4, 0.1 M) containing 0.02% sodium azide was added to different concentrations of flower extract or aminoguanidine (AG) (Sigma-Aldrich, United States), and then bovine serum albumin (BSA) (10 mg/mL) was added and incubated at 37 °C for 4 weeks. The contents of AGEs produced were quantified by spectrofluorometer (excitation wavelength: 355; emission wavelength: 460 nm) and the inhibition rates of the AGEs formation were determined using the following equation:

$$\text{Inhibition rate } (\%) = [1\text{-}((F_S - F_{Sb})/(F_C\text{-}F_{Cb}))] \times 100$$

Fs and Fsb represent the fluorescence intensity of the mixture and the mixture blank (without fructose), respectively. Fc and Fcb correspond to the fluorescence intensity of the control mixture and the control blank mixture, respectively.

2.7.1. Measurement of fructosamine

The fructosamine in glycated materials was determined after 28 days of incubation. Briefly, 40 μL of glycated materials were mixed with 160 μL of 0.3 mM NBT in 100 mM, pH 10.35 sodium carbonate buffer and incubated at 37 °C for 30 min. The absorbance of the mixture was taken at 530 nm. The 1-deoxy-1-morpholino-fructose (1-DMF) (Sigma-Aldrich, United States) was used as a standard curve to calculate the concentration of fructosamine.

2.7.2. Determination of protein thiol group

The level of free thiol in glycated materials was determined after 28 days of incubation using Ellman's reagent. In brief, 250 μL of glycated samples mixture and of 750 μL DTNB solutions (0.5 mM) were mixed and incubated for 15 min at 37 °C. The absorbance was determined at 410 nm using L-cysteine as a standard curve.

2.8. Antidiabetic enzymatic assays

2.8.1. α-amylase inhibitory assay

The inhibition potential of *O. pes-caprae* against α-amylase (Sigma-Aldrich, United States) was performed following a protocol described by Mrid et al. [27]. In brief, 100 μL of *O. pes-caprae* flowers extract prepared in dimethyl sulfoxide (3%) was added to 100 μL of α-amylase solution (0.1 U/mL) prepared in phosphate buffer (pH 6.9) and incubated at 37 °C for 30 min. After pre-incubation, 100 μL of starch solution (0.25%) in phosphate buffer (pH 6.9) was added to each tube. The reaction was carried out at 37 °C for 30 min. Then, the reaction stopped by addition of 200 μL of the DNS reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. The absorbance was measured at 540 nm using a spectrophotometer. Acarbose was used as positive control.

The α-amylase inhibitory activity was expressed by percentage of inhibition that was calculated according to the following equation:

$$\text{Inhibition } (\%) = [((A_C\text{-}A_{Cb})\text{-}(A_S - A_{Sb}))/(A_C\text{-}A_{Cb})] \times 100 \quad (1)$$

where *Ac* is the absorbance of control that was composed of the enzyme; *Acb* refers to the absorbance of control blank (buffer without enzyme); *As* and Asb refers to the absorbance of sample (enzyme and inhibitor) and sample blank (inhibitor without enzyme), respectively.

2.8.2. α-glucosidase inhibition effect

The inhibitory effect of *O. pes-caprae* flowers extract against

α-glucosidase (from yeast, Sigma-Aldrich, United States) was performed as previously described [22,27]. Briefly, 30 μL from each sample prepared in dimethyl sulfoxide (3%) was mixed with 20 μL of sodium phosphate buffer (pH = 6.7) containing 0.1 U/mL of α-glucosidase and incubated at 37 °C for 10 min 40 μL of pNPG (Sigma-Aldrich, United States) solution (1 mM) was added to the mixture and incubated at 37°C for 30 min. Then, 0.1 M of Na_2CO_3 was added and the absorbance was determined by spectrophotometer at 405 nm. Acarbose was used as positive control.

The inhibitory activity was expressed by % inhibition according to the formula (1).

2.9. Experimental animals

The Swiss albino mice of either sex was utilized in the experiment. In accordance with Organization for Economic Cooperation and Development (OECD) guidelines [28], the mice were acclimatized to laboratory conditions with free access to a commercial pellet and water. Before ether anesthesia, the mice were killed and sacrificed at the end of the experiment.

2.9.1. Diabetes induction in experimental animals

After overnight fasted, animals were injected intraperitoneally with a single dose of alloxan monohydrate with a concentration of 150 mg/kg to induce diabetes [29]. Then only animals with blood glucose levels higher than 200 mg/dL, 3 days after injection were designated in the study [30].

2.9.2. Acute toxicity study

The procedure of acute toxicity test was conducted according with the limit test recommended by Guideline 425 of the OECD [28]. The animals were branched into three groups of five mice each and were given the methanolic extract of OPC at the doses of 0 mg/kg (control group), 800 mg/kg, and 2000 mg/kg orally. Mortality and behavioral changes were monitored in the mice during 15 days [31].

2.9.3. Experimental design

The mice were branched into five groups (n = 5) as follows:

Group 1: normal control (non-diabetic),

Group 2: untreated diabetic control,

Group 3: diabetic mice treated by the standard diabetic drug glibenclamide (10 mg/kg),

Group 4 and 5: diabetic mice treated by methanolic flowers extract of *O. pes-caprae* at the dose of 150 and 250 mg/kg, respectively.

The flower extract of *O. pes-caprae* and saline solution were administered via oral gavage during 21 successive days.

2.9.4. Measurements of blood glucose

The blood glucose concentration of each mouse was measured on 1, 7, 14, 21 days, regularly by taking blood from the tail vein using glucose test strips of electronic glucometer (Roche Diabetes Care GmbH, Allemagne) [32].

2.9.5. Body weight analysis

The body weight of each mouse was recorded in 1, 7, 14, 21 days, regularly using a precision balance.

2.9.6. Collection of organs

After anesthetized under mild ether, the mice were dissected. The kidney, liver, and spleen were taken for assessment of biochemical parameters [33].

2.9.7. Antioxidant enzyme assay

The antioxidants enzymatic activities were determined by spectrophotometry. The superoxide dismutase (SOD), glutathione peroxidase activity (GPx) and glutathione reductase (GR) were measured following the method of Ben Mrid et al. [23]. The activity of catalase (CAT) was assayed according to the method of Aebi [34].

2.9.8. Oxidative stress markers

2.9.8.1. Determination of hydrogen peroxide (H_2O_2) level. H_2O_2 level was determined colorimetrically following the protocol of Bouchmaa et al. [35]. Briefly, cells were crushed in 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 12,000*g* for 15 min at 4 °C. The layer was kept in dark for 1 h after mixing with 10 mM of phosphate buffer (pH 7.0) and 1 M of potassium iodide. The absorbance of the resulting solution was measured at 390 nm·H_2O_2 concentrations were calculated using a standard curve.

2.9.8.2. Determination of malondialdehyde (MDA) content. Lipid peroxidation measured as MDA content was determined using thiobarbituric acid (TBA) according to the method described previously by Ohkawa et al. [36] with slight modifications. In brief, in different conditions cell homogenate was mixed with 20% of trichloroacetic acid and 0.67% of TBA. The mixture was heated at 95 °C for 1 h. After cooling, 1 mL n-butanol was added to the mixture followed by centrifugation at 12, 000*g* for 10 min. The absorbance of organic supernatant was measured at 532 nm.

2.9.9. Carbohydrates metabolizing enzymes

Hexokinase was assayed according to the methods of Brandstrup [37]. Pyruvate kinase was determined by the method of Pogson & Denton [38], and the Glucose 6-phosphate dehydrogenase was estimated by the method of Ells & Kirkman [39].

2.9.10. Determination of protein content

Total protein content of the samples was determined following the method of Bradford [40] (Bio-Rad, Hercules, CA, USA) using BSA as a protein standard.

2.10. Statistical analysis

The data represented means values ± standard deviation (SD). All statistics were conducted using one-way analysis of variance (ANOVA) to determine the statistical significance of results ($p < 0.05$), using version 25 of SPSS statistics.

3. Results and discussion

3.1. Polyphenolic composition and antioxidant activity of O. pes-caprae

Polyphenols are a group of secondary metabolites comprising different compounds such as flavonoids, phenolic acids and non-flavonoids [41]. These natural compounds constitute the most effective compounds against oxidative stress, since they have high capacity to scavenge ROS [42]. Additionally, plants with high content of polyphenols can be an excellent source of antioxidants [43], and can be therefore used in diet or used in different preventive or therapeutic applications against human diseases. O. *pes-caprae* plant is known for its high phenolic composition. Hence, in the current study we analysed its phenolic content and determined its antioxidant activity.

While methanol is a perfect solvent for the extraction of polyphenols [40], in a previous study conducted by Halouani et al. [19], the authors showed that water is the best solvent for the extraction of phenolic compounds from O. *pes-caprae* (seeds, stems, leaves and flowers). Moreover, the authors showed that the extraction yields increase from the root (seeds) to the shoot parts (stems, leaves and flowers) [19]. Hence, the extraction yield of a plant depends on its phenolic composition that varies depending on the part of the plant, the appropriate solvent for each compound and the experimental procedure. In our

study, we have analysed total polyphenols and flavonoid contents (TPC and TFC) of O. *pes-caprae* flowers and leaves using both water and methanol as solvents. The data in Table 1 showed that the extraction yield of methanol is higher than that of water, which makes it suitable for the extraction of polar phenolic compounds. The same table shows that aqueous extracts (AE) and methanolic extracts (ME) of *O. pes-caprae* flowers contain high amounts of TPC and TFC compared to those of O. *pes-caprae leaves*.

In addition, the highest amounts of TPC and TFC were found in ME of OPC flowers; with ME of flowers containing 173.5 mg GAE/g dw and 471.6 mg QE/g dw of TPC and TFC, respectively; and AE of flowers containing 112.6 mg GAE/g dw and 315, 5 mg QE/g of TPC and TFC, respectively. Contrariwise, the O. *pes-caprae* leaves extracts showed low amounts of TPC and TFC compared to O. *pes-caprae* flower extracts, with AE of OPC leaves containing 54.303 mg GAE/g Dw and 86.189 mg QE/g Dw of TPC and TFC, respectively; and ME of OPC leaves containing 70.042 mg GAE/g Dw and 114.301 mg QE/g Dw of TPC and TFC, respectively. Similarly, a study conducted by Gul et al. [44], has shown that the ME of OPC flowers have a slightly higher level of phenolic compounds compared with the ME of OPC leaves, with flowers' ME containing 65.68 mg GAE/g of TPC and 24.75 mg QE/g Dw of TFC; and leaves' ME containing 57.04 mg GAE/g of TPC and 23.35 mg QE/g Dw of TFC. Oppositely, another study conducted by Mukherjee et al. [45], showed that the leaf extract of *oxalis corniculate* contained 910 GAE/g dw of TPC, and 2.353 g/100 g dw of TFC. Whereas, the study conducted by Liao et al. [46], revealed that the leaf extract of oxalis *corymbosa* contained 8.74 mg GAE/g of TPC and increased FTC in winter that reach 30 mg retinol equivalent (RE)/g. Hence, the quantity of phenolic compounds differs also depending on the plant species, the geographical regions of growth, and the climatic and seasonal variations. Phenolic compounds (polyphenols and flavonoids) are known to have a high scavenging capacity against ROS [22]. Hence, in order to determine the scavenging capability of our extracts, we have used four different tests DPPH• scavenging, $ABTS^{+}$• scavenging, metal reducing activity, and ferric reducing antioxidant power (FRAP) tests to analyse the scavenging activity of our extracts and provide a reliable data for their antioxidant capacity. Results were expressed by IC_{50}, as shown in Table 1.

Both AE and ME of *O. pes-caprae* flowers showed high trapping DPPH scavenging capacity with an IC_{50} of 0.114 mg/mL and 0.134 mg/mL, respectively.Gallic acid showed an IC_{50} value of 0.009 mg/mL. On the other hand, the ME of flowers showed a high capacity to quench $ABTS^{+}$• with an IC_{50} of 0.297 mg/l compared to the AE of flowers (IC_{50} =0.366 mg/l), but less potent than the quercetin (IC_{50} =0.039 mg/mL). The AE and ME of *O. pes-caprae* leaves had a DPPH scavenging of 0.296 mg/mL and 0.214 mg/mL, respectively; and ABTS scavenging of 0.88 mg/mL and 0.925 mg/mL, respectively. Hence, the scavenging capacity of *O. pes-caprae* flowers is higher than the one of *O. pes-caprae* leaves. This could be explained by the high TPC and TFC in flowers compared with leaves. However, this rule is not always applicable since despite that ME of leaves has high levels of TPC and TFC compared to AE leaves, it has a lower ABTS scavenging capability compared to that of AE leaves (0.925 vs 0.88). This may be due to the presence of compounds that were highly extracted by water compared to methanol, and that have a scavenging capacity different from those present in ME. In addition, it can also be related to the presence or absence of one or more compounds that act in synergy in one of the extracts. Therefore, the scavenging activity of *O. pes-caprae* can vary depending on the amount and type of phenolic compounds present in the extract and the possible synergetic effects between them [18]. Our results showed higher antioxidant activity of O. *pes-caprae* extracts compared to the results obtained by Halouani et al. [19], that showed that the ME of *O. pes-caprae* flowers have a DPPH scavenging capacity of 0.514 ± 0.046 mg/mL and ABTS scavenging capacity of 0.305 ± 0.052 mg/mL. Whereas, AE of *O. pes-caprae* flowers have a DPPH scavenging activity of 0.725 ± 0.006 mg/mL and ABTS scavenging of 0.379 ± 0.0523 mg/mL [19].

High levels of free metals, including iron, in biological systems, constitute an important source of reactive radicals, that may lead to several cellular damages [47]. Therefore, we have analysed the metal chelating activity of our extracts to determine their antioxidant potential. The ME of *O. pes-caprae* flowers had a higher metal chelating activity compared to the AE of O. *pes-caprae* flowers with an IC_{50} of 1.143 mg/mL versus 4.038 mg/mL for the AE, but less potent than the standard EDTA that had and IC_{50} value of 0.296 mg/mL (Table 1).

The FRAP assay is a method used to quantify the reduction of the Fe^{3+}/Ferricyanide complex to the ferrous form (Fe^{2+}) by plant extracts [27]. The results in Table 1 present the reducing power of both ME and AE expressed through Ascorbic acid equivalent (AAE). The highest value of the reducing power was recorded in ME of flowers with a value of 795.8 mg AAE/g dw compared to the AE of flowers (690.8 mg AAE/g dw). In contrast, the ME and AE of leaves presented a lower reducing power of 507.8 mg AAE/g dw and 387.4 mg AAE/g dw, respectively, compared to the ME and AE of flowers. This can be explained by the low amount of phenolic compounds present in *O. pes-caprae* leaves compared to *O. pes-caprae* flowers. Hence, these results demonstrate that *O. pes-caprae* extracts have a reducing power that is dependent on the concentration of phenolic compounds.

O. *pes-caprae* flowers showed potent antioxidant capacity exhibited by its scavenging ability, metal chelation, and reducing power. This activity may be related to the presence of different active phenolic compounds such as polyphenols and flavonoids that have the capacity to absorb and neutralize free radicals [48]. Several other researchers have reported similar results [18,19,49,50]. It was previously demonstrated that the aerial parts of *O. pes-caprae* contain high levels of antioxidant compounds such as luteolin glucoside, cernuoside, as well as chlorogenic acid, quinic fernulate, and luteolin C-O-diglucoside [49,51]. Other Oxalis species have also demonstrated their antioxidant activity. For instance, *O. acetosella* leaves exert antioxidant activity due to the presence of carotene, ascorbic acid and flavonoids [50]. Whereas,

Table 1
Bioactive compounds and antioxidant activity of *O. pes-caprae.*

		TPC (mg GAE /g dw)	TFC (mg QE /g dw)	Antioxidants properties (IC_{50} values)			Reducing power (mg AAE /g dw)
				DPPH (mg/mL)	ABTS (mg/mL)	Metal chelating activity (mg/mL)	
O. pes-caprae leaves	**AE**	54.303 ± 2.259^{Aa}	86.189 ± 4.322^{Aa}	0.296 ± 0.009^{Aa}	0.88 ± 0.007^{Aa}	0.198 ± 0.001^{Aa}	387.433 ± 3.384^{Aa}
	ME	70.042 ± 2.361^{Ab}	114.301 ± 5.904^{Ac}	0.214 ± 0.009^{Ab}	0.925 ± 0.005^{Ac}	$↱0.401 \pm 0.012↱^{Ab}$	507.805 ± 1.717^{Bc}
O. pes-caprae flowers	**AE**	112.6 ± 6.944^{Ba}	315.454 ± 15.266^{Bb}	0.114 ± 0.004^{Ba}	0.366 ± 0.008^{Bb}	4.038 ± 0.207^{Ba}	690.83 ± 11.858^{Cb}
	ME	173.537 ± 12.317^{Bb}	471.657 ± 15.13^{Bc}	0.134 ± 0.023^{Bb}	0.297 ± 0.007^{Bd}	1.143 ± 0.026^{Bb}	795.801 ± 5.101^{Cc}
Gallic acid		-	-	0.009 ± 0.001^{c}	-	-	-
Quercetin		-	-	-	0.039 ± 0.001^{e}	-	-
EDTA		-	-	-	-	0.296 ± 0.001^{c}	-

All values are means ± standard deviation. TPC: total phenolic content. TFC: total flavonoid content. GAE: Gallic Acid Equivalent. QE: Quercetin Equivalent. AAE: Ascorbic acid Equivalent. EDTA: Ethylenediaminetetraacetic acid. IC_{50}: The extract concentration providing 50% inhibition. DPPH: 2,2-diphenyl-1-picrylhydrazyl. ABTS: 2,2′-azino-bis(3ethylbenzothiazoline-6-sulfonic acid). dw: dry weight. AE: Aqueous ↱Extract. ME: Methanolic Extract. Different uppercase letters in the column indicate significant differences between extracts and lowercase letters in the column indicate significant differences between part of plant ($p < 0.05$).

O. corniculata have an antioxidant activity arising from the presence of high content of vitamin c, lycopene, phenolic compounds, beta carotene and flavonoids [52]. Hence, further studies should be conducted to characterize the compounds present in our extracts and the specific activity of each of it. Moreover, DM2 is characterized by a metabolic disorder that induces high generation of ROS [51]. The imbalance between ROS production and the function of antioxidant proteins leads to a continued oxidative stress. This oxidative stress affects proteins, lipids and nucleic acids and damages cellular structure and functions in the organism [51]. It also exacerbates the evolution of diabetes and the development of a wide range of complications including retinopathy, neuropathy, coronary artery disease, etc [51,53]. Additionally, low grade inflammation can engender DM2 due to its implication in the induction of insulin resistance. In addition, hyperglycaemic-induced oxidative stress increases inflammation, and inflammation in its turn increases the release of ROS [54]. Hence, the studied extracts can have a relevant use for the encountering of ROS levels and the restoration of redox homeostasis in order to prevent further DM2 complications. However, proof-of-concept are needed to identify the compound(s) responsible(s) of the antioxidant activity of our extracts, their mechanism of action and their efficacy in vivo.

The phenolic profile of ME of *O. pes-caprae*, attained by HPLC-DAD-ESI/MS analysis, is displayed in Fig. 1. Peak identification is reported in Table 2. The most abundant compound was iso-luteolin-*O*-dihydrogalloyl-trihexoside (peaks no. 3–5–6–9–12), followed by kaempferol-*O*-coumaroyl hexoside (peak no. 8). Other compounds are identified in extract such as quinic acid (peak no. 1), isovitexin-*O*-hexoside (peak no. 11), apigenin-di-C-hexoside (peak no. 13) and quercetin-*O*-hexoside (peak no. 16).

3.2. *In vitro study of anti-diabetic activities of O. pes-caprae flowers extract*

α-amylase and α-glucosidase represent two important enzymes responsible for the breakdown of carbohydrates into glucose. Inhibition of these two enzymes is among the strategies used to control hyperglycemia and treat DM2 [27]. Acarbose is the common drug used to treat DM2 by inhibiting these two enzymes [28]. However, acarbose is a synthetic drug and can lead to several undesirable effects like diarrhea, stomach pain, and digestion difficulties [29]. In the present study, the antidiabetic activity of *O. pes-caprae* flower through the analysis of its inhibitory activity against α-amylase and α-glucosidase enzymes, was examined. The results presented in Fig. 2 show that the ME of *O. pes-caprae* flowers had an important inhibitory effect on both α-amylase and α-glucosidase. The inhibitory activity of ME against α-glucosidase was 92.14% at a concentration of 890 μg/mL, while the inhibitory activity of acarbose was 50% at a concentration of 330 μg/mL (Fig. 2A). Additionally, ME inhibited the α-amylase activity by 67.857% at a concentration of 187.50 μg/mL, while acarbose inhibited 50% of α-amylase at a concentration of 13.64 μg/mL (Fig. 2B). From these results, it was clear that the ME of O. *pes-caprae* flowers contain molecules that could play the role of inhibitors of both α-glucosidase and α-amylase in a dose dependent manner. Moreover, at a concentration of 187.50 μg/mL, the investigated extract inhibited 68% of α-amylase. Whilst, at 222.50 μg/mL, it inhibited 40% of α-glucosidase. This may be due to the different interactions and affinities between the compounds and the active site residues of each enzyme. Previous studies have linked the richness of plants on flavonoids such as those present in our methanolic extract of *O. pes-caprae* flower (471.657 mg QE /g dw) to the potential to inhibit α-amylase and α-glucosidase [55–57]. In these studies it was reported that some flavonoids, in particular quercetin, luteolin, and eriodictyol are natural inhibitors of α-amylase and α-glucosidase due to their ability to bind non-covalently into the active site residues of these enzymes. However, to validate these hypotheses, the active compounds should be isolated from the flowers of *O. pes-caprae* in order to evaluate the mechanisms by which they act on the two enzymes.

3.3. *Inhibition of O. pes-caprae flowers extract on non-enzymatic glycation process*

The major consequence of hyperglycemia is a non enzymatic glycation reaction caused by excessive cross-links of reducing sugars to the amino groups of proteins. The initial stage of this process involves the condensation between monosaccharides such as glucose, fructose, or ribose and the terminal amino groups of proteins, nucleic acids, or lipids. This interaction lead to the formation of early glycation products, also known as Amadori products including fructosamine [58,59]. Subsequently, the Amadori products react with amino acids and lead to the formation of complex compounds called advanced glycation end products (AGEs). The accumulation of AGEs which alters intracellular and extracellular function is a responsible factor for the long-term complications of DM2 [58,60]. Thus, the inhibition of the formation of AGEs and their intermediates is essential for the prevention of DM2 complications. In the present study, the inhibitory effect of ME of *O. pes-caprae* flowers on glycation and the formation of AGEs was studied using an in

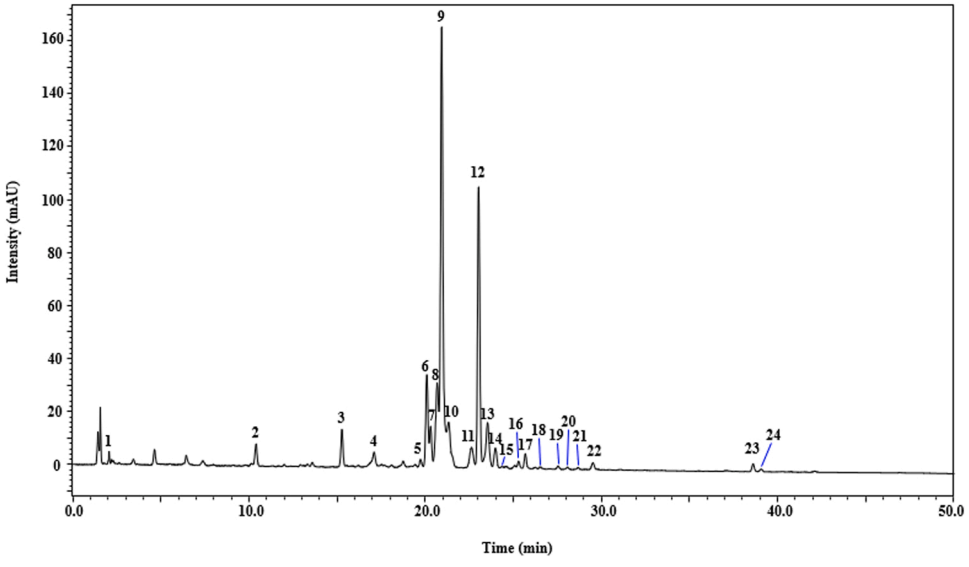

Fig. 1. Chromatogram of phenolic compounds in ME of *O. pes-caprae* detected at 280 nm.

Table 2
Characterization of phenolic compounds of *O. pes-caprae* extract by HPLC-PDA/ESI-MS. Values represent averages of phenolic compounds in extract (mg/kg ± sd) of triplicate measurements. Column: Ascentis Express C18, 15 cm × 4.6 mm, 2.7 μm d.p. (ESI, negative ionization mode; when observed, secondary fragment ions are reported).

Peak	Compound	t_R (min)	UV_{max} (nm)	$[M-H]^-$ *m/z*	MS fragments	*O. pes-caprae* extract mg/Kg ± sd
1	Quinic acid	2.05	-	191	111	-
2	Luteolin-*O*-dihydrogalloyl-trihexoside	10.38	289	895	447, 327, 285	1.71 ± 0.021
3	Iso-luteolin-*O*-dihydrogalloyl-trihexoside	15.25	293	895	447, 327, 285	3.80 ± 0.079
4	Kaempferol-di-*O*-hexoside	17.06	346	609	519, 447, 327, 297	11.65 ± 0.133
5	Iso-luteolin-*O*-dihydrogalloyl-trihexoside	19.71	327	895	447, 327, 285	3.64 ± 0.082
6	Iso-luteolin-*O*-dihydrogalloyl-trihexoside	20.07	325	895	447, 285	48.05 ± 0.171
7	Kaempferol-di-*O*-hexoside	20.29	347	609	447, 327, 299, 285	16.71 ± 0.065
8	Kaempferol-*O*-coumaroyl hexoside	20.65	349	609	593, 327, 309	51.79 ± 0.142
9	Iso-luteolin-*O*-dihydrogalloyl-trihexoside	20.92	348	895	483, 446, 327, 297, 357	245.48 ± 0.263
10	Kaempferol-3-apiosyl-hexoside	21.31	347	579	447, 357, 327, 309, 285	33.59 ± 0.113
11	Isovitexin-*O*-hexoside	22.59	341	593	293	7.25 ± 0.033
12	Iso-luteolin-*O*-dihydrogalloyl-trihexoside	23.01	330, 401	895	447, 341, 285	166.12 ± 0.181
13	Apigenin-di-C-hexoside	23.51	341	577	563, 431, 327, 297, 283	15.24 ± 0.041
14	Iso-apigenin-di C-hexoside	23.97	268, 341	609	431, 473, 298, 284	5.11 ± 0.0163
15	Iso-apigenin-di-C-hexoside	24.33	397	563	431, 447, 285	1.14 ± 0.014
16	Quercetin-*O*-hexoside	25.05	346	463	-	0.73 ± 0.010
17	Kaempferol-pentose	25.65	346	417	285, 284	7.80 ± 0.064
18	Kaempferol-di-hexoside	26.50	346	609	285, 297	1.11 ± 0.086
19	Unknown	27.51	402	558	161, 269, 322	-
20	Unknown	28.05	395	563	431, 383, 293	-
21	Luteolin	28.65	265, 346	447	-	0.86 ± 0.056
22	Kaempferol-hexoside	29.48	328, 400	447	285	4.99 ± 0.061
23	Unknown	38.59	316	614	478, 332	-
24	Kaempferol	39.07	346	285	-	1.59 ± 0.012

vitro BSA-fructose model. Aminoguanidine (AG), an antiglycation agent that inhibits the formation of AGEs was used as a control. To our knowledge, the antiglycation activity of *O. pes-caprae* flower extract has not yet been.

The results presented in Fig. 3 show the anti-glycation capacity of *O. pes-caprae* flower extract at different concentrations and at different stages of the non-enzymatic glycation process. Fig. 3A shows the amount of fructosamine produced after 4 weeks in the mixtures of BSA-fructose with *O. pes-caprae* flower extract or AG. The results demonstrateda reduction in the fructosamine levels in a dose-dependent manner. The amount of fructosamine was decreased from 2.12 mM to 1.16 mM as the concentration of *O. pes-caprae* flower extract increased from 0.06 mg/mL to 1 mg/mL. Compared to positive control with 1.5 mM of fructosamine, the flowers extract had a higher inhibitory effect on the formation of early-stage glycation products.

Furthermore, thiols are important chemical groups in the organism. They contain sulfhydryl groups (-SH) which several antioxidant enzymes can use to increase their antioxidant activity, especially glutathione peroxidase [61]. It is known that thiols have the capacity to receive excess electrons and stabilize free radicals to prevent oxidative stress-induced damage [61]. In addition, thiols play a key role in various enzymatic reactions. They are involved in transcription, signal transduction, cellular signaling, detoxification, apoptosis, etc [61]. However, these thiol groups are prone to oxidation and glycation events. On the one hand, thiol glycation can generate protein cross-linking, which promotes DM2 complications such as vascular diseases [62]. On the other hand, the oxidation of antioxidant enzymes thiol groups results in a loss of their antioxidant function, which increases oxidative stress and the progression of DM2 [63]. Therefore, the inhibition of glycation/oxidation of thiols is important for the prevention of DM2 complications. Fig. 3. B shows the effects of *O. pes-caprae* flower extract on the glycation of protein thiols. After incubation, the mixture of BSA and fructose had low levels of free thiol groups compared to the BSA alone, which confirmed the glycation of protein thiol. In contrast, the levels of protein thiols increased from 0.28 to 2.41 nmoL/mg once the concentration of ME increased from 0.06 to 1 mg/mL. In addition, at a concentration of 1 mg/mL, the ME increased protein thiols to 2.41 nmoL/mg compared to the control (AG) that increased it to a shorter level of 0.6 nmol/mg at the same concentration. Hence, *O. pes-caprae* extract could be a potential glycation inhibitor that has the capacity to prevent the glycation of antioxidant proteins and the loss of their function as well as restores redox homeostasis in DM2 patients.

The irreversible formation of AGEs during the last stage of glycation process has a negative consequence on the progression of DM2 [64]. They interact with AGE-specific receptor (RAGE) which is implicated in the pathogenesis and complications of DM2, causing increased generation of ROS and the activation of inflammation [64]. Interestingly, RAGE is expressed on multiple immune cells such as T cells, monocytes, and macrophages. It enhances pro-inflammatory cytokines production affiliated with cellular stress and damage in different cells notablyglial cells, neurons, macrovessel endothelium, etc, leading to the development of DM2 complications [64]. Addionally, RAGE activates NADPH oxidase-mediated ROS production, leading to ER stress, inflammation, podocyte injury, and renal fibrosis [65]. Hence, we evaluated the inhibitory effect of our extracts on AGEs formation. Fig. 3C shows that *O. pes-caprae* flower extract inhibited AGEs formation in a dose-dependent manner. The formation of AGEs in the mixtures of BSA-fructose and flower extract or AG was reduced after 4 weeks of incubation. The highest inhibitory rate (99.45%) was recorded at 1 mg/mL of flowers extract, while, AG inhibited the formation of AGE by 89.2% at the same concentration.

In the present investigation, we showed that *O. pes-caprae* extract acts at different stages of glycation and reduces significantly the formation of early and advanced glycation end products. The inhibitory activity of *O. pes-caprae* could be attributed to polyphenols that were previously approved to inhibit AGE formation through different mechanisms [66]. Firstly, they allow the scavenging of oxidative free radicals. Secondly, they cover the glycation sites of proteins (e.g. Thiol groups). Thirdly, they trap active dicarbonyl compounds. And fourthly, they inhibits chelating metal ions [66]. All of these mechanisms were confirmed in our study. And *O. pes-caprae* extracts have demonstrated their efficacy in scavenging free radicals, covering the sites of protein thiols, inhibiting metal chelation, and also reducing hyperglycemia via the inhibition of α-amylase and α-glucosidase. All of these mechanisms

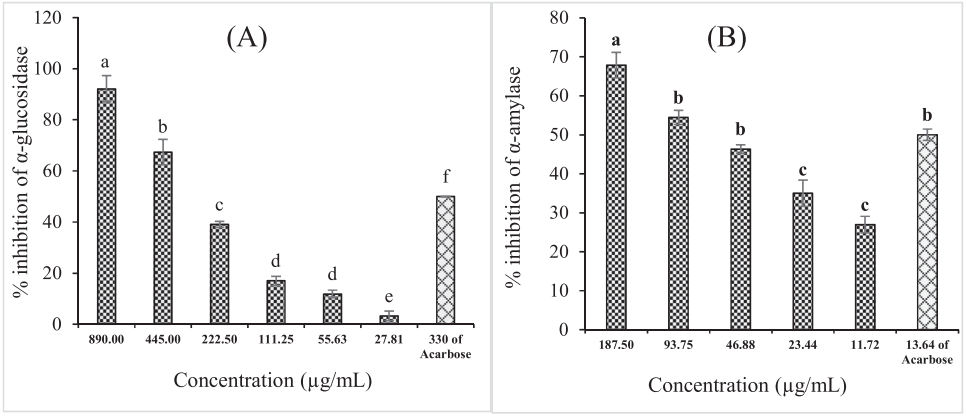

Fig. 2. Percentage of α-glucosidase inhibition (A) and α-amylase inhibition (B) versus different concentrations of *O. pes-caprae* flowers extract and acarbose. Each value represents the mean of three replicates. Bars represent the standard error. Different letters indicate significant differences among treatments at $P < 0.05$.

lead to the reduction of glycation and oxidation and consequently reverse DM2. Other secondary metabolites can also inhibit AGEs formation such as terpenoids, alkaloids, and vitamins. Hence, further characterization of *O. pes-caprae's* composition should be conducted. Moreover, the inhibitory activity of *O. pes-caprae* could also be attributed to flavonoids. A study conducted by Wu. et al., have demonstrated that the inhibitory activity of flavonoids against protein glycation is attributed to its scavenging capability against free radicals, which was approved in our study [66]. In addition, it was demonstrated that many flavonoid compounds such as apigenin, chrysin, kaempferol, myricetin, pinocembrin, diosmetin, liquiritin, ECGC, proanthocyanidins, phlorein, calyosin, etc decrease AGE-induced inflammation and ROS production in vitro and in vivo [67].

3.4. Acute toxicity study

Compared to safety data, the acute toxicity test at an oral limit dose of 800 mg/kg and 2000 mg/kg of O. *pes-caprae* flowers ME caused no changes in mice behavior and no lethality during the 15 days of observation. Therefore, the extract may be safe at these doses, and the oral LD_{50} was greater than 2000 mg/kg body weight in mice.

3.5. Effect of O. pes-caprae ME on body weight and fasting blood glucose level in diabetic mice

After induction of diabetes in mice through the introduction of 150 mg/kg of alloxan, the effects of OPC flowers extract on body weight and fasting blood glucose were monitored (Table 3 and Fig. 4). Normal control group (non-diabetic mice) had a gain of 27.12% of their body weight. However, this gain is statically significant. Diabetic mice had a significant loss of 27% of their body weight between day 0 to day 21. Mice treated with 250 mg/kg of OPC ME showed an increase of 25% of their body weight. Whereas, those treated with 150 mg/kg of OPC ME or 10 mg/kg of glibenclamide showed an increase of their body weight from day 0 to day 21. Al-Attar & Zari [68] showed that the body weight of mice indicated the efficacy of antidiabetic treatment. The decrease of body weight may be associated with the metabolism change in diabetic mice. Increased hyperglycemia with low glucose intake due to insulin resistance induces an activation of gluconeogenesis, glycogenolysis, amino acid metabolism and lipolysis for energy production [69]. Hence, this may explain the decrease in fat levels.

Moreover, all of glibenclamide at a concentration of 10 mg/kg and OPC ME at a concentration of 150 mg/kg and 250 mg/kg decreased fasting blood glucose to an amount inferior than 200 mg/dl after 3 weeks of treatment. The decrease of blood sugar after treatment with OPC ME or glibenclamide could be associated to the restoration of normal metabolism through the stimulation of lipogenesis and consequently, the increase of body weight.

3.6. Effect of methanolic extract of OPC flowers on the activities of antioxidant enzymes in mice

The excess of ROS generated in DM2 is encountered by an intracellular antioxidant defense mechanism. This defense mechanism relies on the presence of antioxidant enzymes such as superoxide dismutase (SOD), catalases (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) that catalyze the decomposition of ROS and maintain redox homeostasis. SOD is considered the primary barrier against ROS which catalyzes the conversion of superoxide anion ($O_2^{-\bullet}$) to hydrogen peroxide (H_2O_2) and oxygen [70]. The generated H_2O_2 can be neutralized to H_2O principally by CAT and GPx, which protects the lipids of cell membranes against oxidative damage [71]. In addition, the reduction of GSSG to two molecules of GSH catalyzed by GR leads to the formation of a large amount of GSH, which is involved in the metabolic pathways of protection under stress conditions and also in the reactions of biomass energy production [72]. However, the high levels of glucose in DM2 lead to the glycation of proteins including those implicated in the antioxidant defense such as SOD, CAT, GPx, and GR, which alter their antioxidant function.

In alignment with these ascertainments, we have analyzed the activities of these four antioxidant enzymes in normal mice, untreated diabetic mice, and diabetic mice treated with either 10 mg/kg of glibenclamide, 150 mg/kg OPC ME, and 250 mg/kg OPC ME. The results obtained in the present study demonstrated that the activities of antioxidant enzymes including SOD, CAT, GPx and GR present in liver, kidney and spleen, decrease significantly in diabetic mice group compared to normal mice group. Our results are correlated with several studies that indicated that the activities of these enzymes are significantly decreased in diabetic patients [73]. Hence, hyperglycemia enhances oxidative stress in these detoxification organs through the impairment of antioxidant enzymes. Moreover, the activities of these antioxidant enzymes significantly increased in different organs of diabetic mice after the treatment by ME of OPC at the doses of 150 mg/kg and 250 mg/kg, indicating the efficacy of OPC ME in the attenuation of the oxidative stress. In addition, treatment of mice with glibenclamide at the dose of 10 mg/kg also increased the activities of the studied enzymes (Table 4).

The alteration of antioxidant enzymes functions increases the levels of ROS in diabetic patients. Hence, to determine the level of oxidative stress in the different organs of the studied mice groups, we analyzed H_2O_2 content and malondialdehyde (MDA) levels. Results in Fig. 5 demonstrates that the levels of H_2O_2 in diabetic mice are higher than normal mice. These levels decreased significantly in diabetic mice treated by ME of OPC compared to untreated mice (Fig. 5A). Treatment

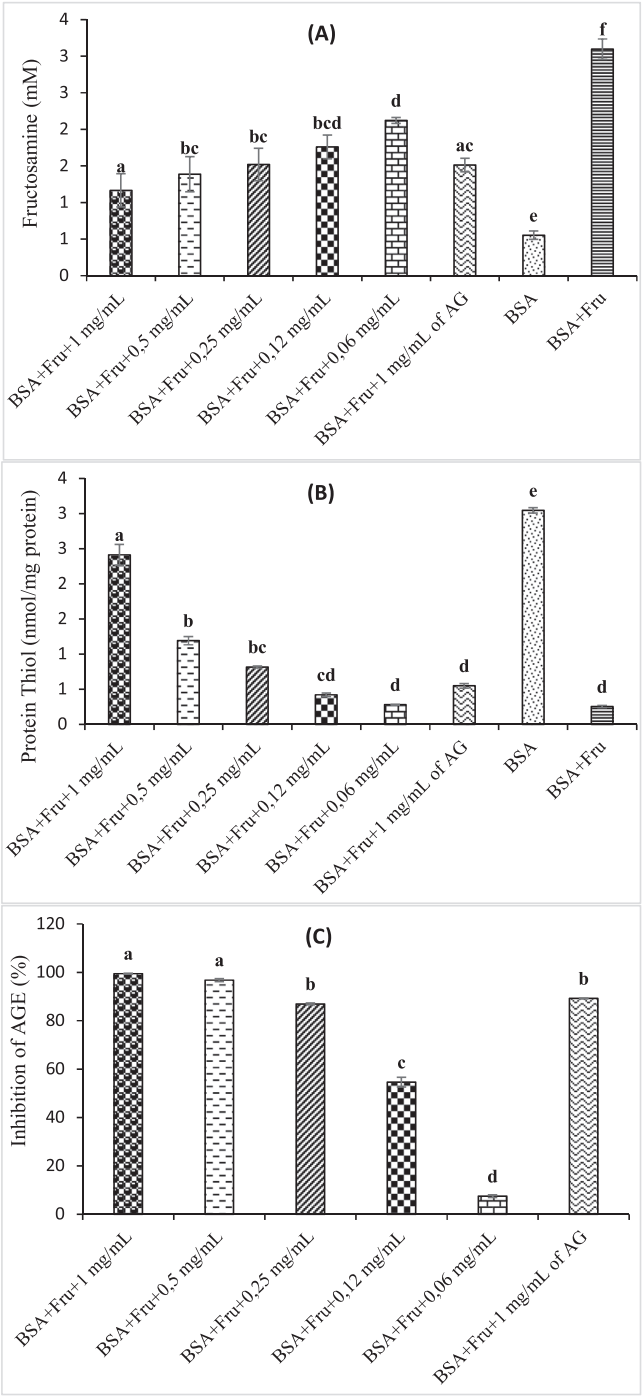

Fig. 3. The effects of *O. pes-caprae* flower extract and aminoguanidine on (A) the percentage of AGE inhibition in BSA/fructose system, (B) the level of fructosamine and (C) the level of protein thiol group. Each value represents the mean of three replicates. Bars represent the standard error. Different letters indicate significant differences among treatments at $p < 0.05$.

with glibenclamide also decreased significantly H_2O_2 content. Therefore, the decrease of H_2O_2 levels could be attributed to the reactivation of antioxidant enzymes by OPC extracts.

The interaction of H_2O_2 with free metals generates hydroxyl radical (•OH), the most reactive and hazardous radical [74]. This •OH is implicated in lipid peroxidation. It oxidizes lipids mainly polyunsaturated fatty acids (PUFAs) that consequently produce various reactive intermediates such as MDA [74]. MDA remains the final product of PUFAs. It is a cytotoxic product indicator of lipid

Table 3
Effect of *O. pes-caprae* (*OPC*) flowers extract on body weight of mice.

Treatments	Dose (mg/kg)	Body weight (g)			
		Day 0	Day 7	Day 14	Day 21
Normal control	-	23.1 ± 2.02[a]	28.3 ± 2.7[a]	28.9 ± 0.56[a]	31.7 ± 0.92[a]
Diabetic control	-	23.7 ± 1.12[a]	20.7 ± 0.38[b]	18.5 ± 1.55[b]	17.3 ± 0.87[b]
Glibenclamide	10	24.5 ± 2.46[a]	27.6 ± 3.78[c]	28.5 ± 1.81[c]	26.6 ± 1.41[c]
OPC flowers extract	150	28.7 ± 1.43[b]	31.5 ± 3.97[d]	33.1 ± 3.96[d]	30.3 ± 0.83[d]
OPC flowers extract	250	22.4 ± 0.26[c]	21.8 ± 0.11[e]	25.4 ± 2.99[e]	28.1 ± 2.34[e]

All the data have been expressed in mean±SD. Different alphabets in the same column indicate significant differences according to tukey's multiple range tests $P < 0.05$ compared with diabetic control.

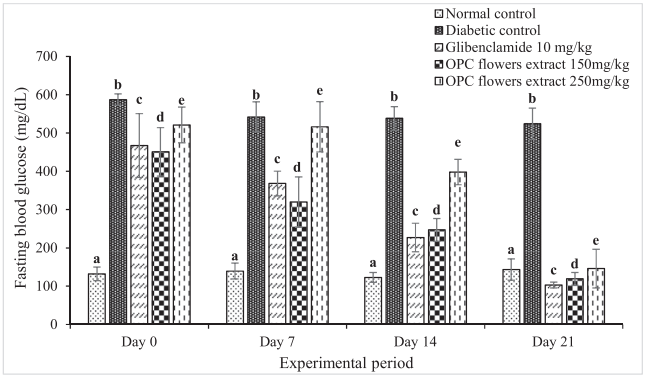

Fig. 4. Effect of the continuous administration of *O. pes-caprae* (OPC) flowers extract on fasting blood glucose on alloxan-induced diabetic mice for 3 weeks. Bars represent the standard error. Different alphabets indicate significant differences according to tukey's multiple range tests $P < 0.05$ compared with diabetic control.

peroxidation and a biomarker of oxidative stress [74]. Its cytotoxicity arises from its high reactivity towards proteins, lipoproteins and DNA, which can induce the development of DM2 complications such as atherosclerosis [75]. In addition, its high levels indicate an increased lipid peroxidation in DM2, which is linked to enhanced pro-inflammatory cytokine production including IL-1β, TNF-α and consequently high risk of endothelial dysfunction and atherosclerosis development [74,76].

In this study, we observe that MDA content is elevated in kidney, liver and spleen of diabetic mice compared to normal control. The methanolic extract induced a slight decrease in MDA content in the liver, kidney, and spleen of diabetic mice group (Fig. 5B). Similarly, the mice group treated with glibenclamide at the dose of 10 mg/kg showed a decrease in MDA content.

3.7. Effects of methanolic extract of OPC flowers on carbohydrate metabolic enzymes in mice

To analyze the antidiabetic activity of OPC extract, we have evaluated their effects on the activities of enzymes involved in carbohydrate metabolism including hexokinase (HK), glucose-6-phosphate dehydrogenase (G6PD), and pyruvate kinase (PK). The HK is the initial enzyme of glycolysis that catalyzes the phosphorylation of glucose to glucose-6-P [77]. The G6PD catalyzes the first step of pentose phosphate pathway [78]. The PK is the enzyme involved in the last step of glycolysis. It catalyzes the transfer of phosphate from phosphoenolpyruvate (PEP) to ADP resulting in the production of pyruvate and ATP [79]. The activities of these enzymes in the liver, kidney, and spleen of normal mice group,

Table 4
Effect of *O. pes-caprae* flowers extract on the activities of antioxidant enzymes in alloxan induced diabetic mice.

Parameters	Samples	Normal control	Diabetic control	Glibenclamide 10 mg/kg	OPC flowers extract 150 mg/kg	OPC flowers extract 250 mg/kg
SOD	Liver	4.53 ± 0.18^{a}	2.63 ± 0.22^{b}	3.68 ± 0.001^{c}	3.16 ± 0.04^{d}	3.11 ± 0.03^{d}
μmol min^{-1} mg^{-1} prot	kidney	7.89 ± 0.73^{a}	1.3 ± 0.14^{b}	6.67 ± 0.02^{c}	5.75 ± 0.31^{c}	3.62 ± 0.01^{d}
	spleen	6.16 ± 0.02^{a}	4.48 ± 0.39^{b}	5.8 ± 0.02^{a}	5.79 ± 0.07^{a}	5.26 ± 0.04^{c}
CAT	Liver	16.04 ± 0.38^{a}	2.92 ± 0.43^{b}	12.47 ± 2.17^{a}	5.89 ± 1.03^{b}	5.24 ± 0.14^{b}
μmol min^{-1} mg^{-1} prot	kidney	16.37 ± 0.66^{a}	8.71 ± 0.16^{b}	10.89 ± 0.45^{c}	10.22 ± 0.62^{c}	9.61 ± 0.31^{bc}
	spleen	8.09 ± 0.16^{a}	6.52 ± 0.48^{b}	7.69 ± 0.73^{ab}	6.74 ± 0.28^{bc}	7.85 ± 0.25^{ac}
GPx	Liver	19.32 ± 0.3^{a}	15.26 ± 0.34^{b}	16.46 ± 0.19^{c}	17.23 ± 0.55^{c}	19.89 ± 0.04^{a}
μmol min^{-1} mg^{-1} prot	kidney	20.34 ± 0.12^{a}	18.87 ± 0.18^{b}	19.81 ± 0.12^{b}	19.51 ± 0.09^{c}	19.27 ± 0.25^{c}
	spleen	21.34 ± 0.05^{a}	16.24 ± 0.09^{b}	17.34 ± 0.15^{c}	20.26 ± 0.09^{d}	19.64 ± 0.19^{e}
GR	Liver	8.02 ± 0.32^{a}	4.21 ± 0.4^{b}	7.72 ± 0.29^{ac}	6.29 ± 0.68^{d}	6.87 ± 0.34^{cd}
μmol min^{-1} mg^{-1} prot	kidney	15.69 ± 0.49^{a}	10.36 ± 0.79^{b}	14.52 ± 0.61^{c}	13.61 ± 0.16^{c}	13.68 ± 0.37^{c}
	spleen	14.15 ± 0.44^{a}	12.43 ± 0.42^{b}	13.97 ± 0.41^{a}	13.52 ± 0.11^{a}	13.14 ± 0.75^{ab}

All the data have been expressed in mean±SEM. SOD: superoxide dismutase. CAT: Catalase. GPx: glutathione peroxidase. GR: glutathione reductase. Different letters in the line indicate significant differences among treatments at $p < 0.05$.

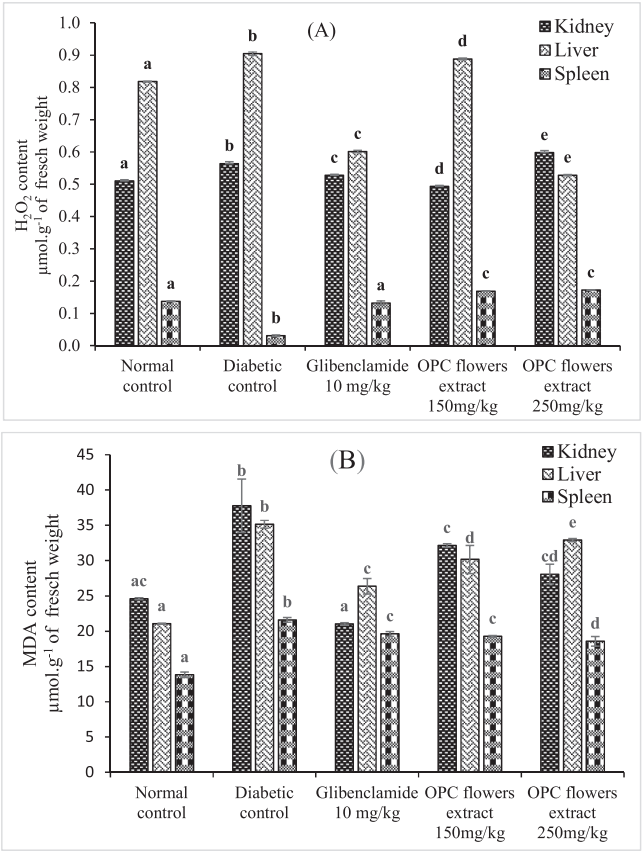

Fig. 5. H_2O_2 content (A) and MDA content (B) in alloxan induced diabetic mice. Bars represent the standard error. Different letters in the line indicate significant differences among treatments at $p < 0.05$.

alloxan-induced diabetic mice, and diabetic mice treated with ME of OPC flowers are shown in Table 5. The activities of HK, PK, and G6PD were significantly reduced in the different organs of diabetic mice group (In liver for example: 0.27 ± 0.02; 2.03 ± 0.04; 0.25 ± 0.01, respectively) as compared to the normal mice group (In liver for example: 0.61 ± 0.03; 3.12 ± 0.14; 0.51 ± 0.08, respectively). Since the mechanism of the induction of DM2 in mice by alloxan involves the partial degradation of beta cells of pancreatic islets and the reduction of insulin production by these cells [80]. The decreased activity of carbohydrate enzymes may be a consequence of low insulin production. A decrease in insulin production activates glucose production and decreases the activation of enzymes involved in glycolysis [81]. The administration of OPC ME and glibenclamide to diabetic mice increased significantly the activities of HK, PK, and G6PD. The restoration of carbohydrate enzymes activities could be attributed to the reduction of blood sugar levels due to the inhibition of α-amylase and α-reductase by OPC extracts. Reduced postprandial hyperglycemia reestablishes metabolism homeostasis. Moreover, previous studies have demonstrated that polyphenols can improve insulin resistance through the reduction of postprandial glucose, the regulation of glucose transport, the protection against damage to insulin-secreting pancreatic β cells, and the modulation of insulin signaling pathways [82]. Hence, the reactivation of the studies enzymes could be attributed to the presence of polyphenolic compounds in OPC extract that regulate the glucose metabolism in diabetic mice. However, the level of restoration of enzymatic activities varies depending on the concentration of phenolic compounds in the extract, the type of the enzyme, and the studied organ (Table 5). Therefore, further studies need to be conducted to explore the precise molecular mechanism of OPC extract for its antidiabetic effect and to isolate the antidiabetic bioactive compounds.

4. Conclusions

The present research study showed the richness of *O. pes-caprae* flowers on bioactive compounds. In the terms of antioxidant, antiglycation and antidiabetic, the results showed significant activities. The methanolic extract of *O. pes-caprae* flowers reduced significantly both α-amylase and α-glucosidase activities and a protectives property against protein glycation. Moreover, the administration of the dose 150 mg/kg and 250 mg/kg from methanolic extract of *O. pes-caprae* flowers was able to reduce diabetes in alloxan-induced diabetic mice after 3 weeks of treatment compared with glibenclamide indicating that the phenolics compounds present in methanolic extract have significant antihyperglycemic activity. Hence, these results validate the potency of *O. pes-caprae* as an antidiabetic agent and suggests it could serve as an alternative remedy in ameliorating or protecting against diabetes and its complications.

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CRediT authorship contribution statement

Imad Kabach: Conceptualization, Investigation, Visualization, Writing – original draft. **Najat Bouchmaa:** Conceptualization, Visualization, Investigation, Writing – original draft. **Zakia Zouaoui:** Investigation, Software. **Abdelhamid Ennoury:** Investigation, Visualization. **Sara El Asri:** Investigation, Validation. **Abdelmounaim Laabar:** Investigation, Visualization. **Loubna Oumeslakht:** Investigation, Software. **Francesco Cacciola:** Writing – review & editing, Resources,

Table 5
Effects of *O. pes-caprae* flowers extract on the activities of some glycolysis enzymes in alloxan induced diabetic mice.

Parameters	Samples	Normal control	Diabetic control	Glibenclamide 10 mg/kg	OPC flowers extract 150 mg/kg	OPC flowers extract 250 mg/kg
Hexokinase μmol min^{-1} mg^{-1} prot	Liver	0.61 ± 0.03^{a}	0.27 ± 0.02^{b}	0.41 ± 0.04^{c}	0.6 ± 0.05^{c}	0.63 ± 0.03^{a}
	kidney	1.10 ± 0.05^{ac}	0.84 ± 0.02^{b}	1.21 ± 0.07^{c}	1.01 ± 0.06^{a}	0.99 ± 0.05^{ab}
	spleen	0.93 ± 0.04^{a}	0.57 ± 0.03^{b}	0.83 ± 0.03^{c}	0.59 ± 0.04^{b}	0.78 ± 0.03^{c}
Pyruvate kinase μmol min^{-1} mg^{-1} prot	Liver	3.12 ± 0.14^{ac}	2.03 ± 0.04^{b}	3.46 ± 0.2^{a}	3.44 ± 0.26^{a}	2.69 ± 0.35^{bc}
	kidney	20.01 ± 0.05^{a}	3.23 ± 0.05^{b}	14.64 ± 0.54^{c}	19.35 ± 1.03^{a}	14.41 ± 1.2^{c}
	spleen	28.33 ± 1.02^{a}	2.34 ± 0.21^{b}	12.14 ± 1.18^{c}	24.74 ± 0.35^{d}	10.35 ± 0.08^{c}
Glucose-6-Phosphatase Dehydrogenase μmol min^{-1} mg^{-1} prot	Liver	0.51 ± 0.08^{a}	0.25 ± 0.01^{b}	0.65 ± 0.05^{a}	0.61 ± 0.12^{a}	0.65 ± 0.04^{a}
	kidney	0.52 ± 0.02^{a}	0.22 ± 0.02^{b}	0.48 ± 0.03^{a}	0.57 ± 0.07^{ac}	0.66 ± 0.05^{c}
	spleen	0.51 ± 0.04^{a}	0.36 ± 0.01^{b}	0.51 ± 0.03^{ac}	0.43 ± 0.01^{d}	0.44 ± 0.01^{cd}

All the data have been expressed in mean±SEM. Different letters in the line indicate significant differences among treatments at $p < 0.05$.

Supervision. **Yassine Oulad El Majdoub:** Investigation. **Luigi Mondello:** Writing – review & editing. **Abdelmajid Zyad:** Project administration, Supervision. **Naima Nhiri**: Writing – review & editing **Mohamed Nhiri:** Project administration, Supervision. **Reda Ben Mrid:** Conceptualization, Investigation, Validation, Visualization, Writing – original draft.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

[1] I.D. Federation, IDF Diabetes Atlas 10th International Diabetes Federation, 2022.
[2] A. Singh, R. Kukreti, L. Saso, et, S. Kukreti, Mechanistic insight into oxidative stress-triggered signaling pathways and type 2 diabetes, Molecules vol. 27 (3) (2022) 950.
[3] A.T. Kharroubi et, H.M. Darwish, Diabetes mellitus: the epidemic of the century, World J. Diabetes vol. 6 (6) (2015) 850.
[4] J.E. Okokon, K. Davies, L. John, K. Iwara, W.-W. Li, P.S. Thomas, et al., Phytochemical characterization, antihyperglycaemic and antihyperlipidemic activities of Setaria megaphylla in alloxan-induced diabetic rats, Phytomedicine vol. 2 (1) (2022), 100182.
[5] A. Gupta, et al., Exploring the recent molecular targets for diabetes and associated complications, Mol. Biol. Rep. vol. 48 (2021) 2863–2879.
[6] V.P. Singh, A. Bali, N. Singh, A.S. Jaggi, et al., Advanced glycation end products and diabetic complications, Korean J. Physiol. Pharmacol. Off. J. Korean Physiol. Soc. Korean Soc. Pharmacol. vol. 18 (1) (2014) 1.
[7] R.A. Kowluru, G. Mohammad, et al., Epigenetic modifications in diabetes, Metabolism vol. 126 (2022), 154920.
[8] H. Cui, et al., Antidiabetic medications and the risk of prostate cancer in patients with diabetes mellitus: a systematic review and meta-analysis, Pharmacol. Res. (2022), 106094.
[9] M. Shanmugasundaram, J.E. Pineda, S. Murugapandian, et al., Glucose-lowering medications and Cardiovascular Outcomes, Curr. Cardiol. Rep. vol. 23 (2021) 1–8.
[10] A. Gupta, R. Kumar, A.K. Pandey, et al., Antioxidant and antidiabetic activities of Terminalia bellirica fruit in alloxan induced diabetic rats, S. Afr. J. Bot. vol. 130 (2020) 308–315.
[11] C.I. Chukwuma, M.G. Matsabisa, M.A. Ibrahim, O.L. Erukainure, M.H. Chabalala, M.S. Islam, et al., Medicinal plants with concomitant anti-diabetic and anti-hypertensive effects as potential sources of dual acting therapies against diabetes and hypertension: a review, J. Ethnopharmacol. vol. 235 (2019) 329–360.
[12] Y.P. Naveen, A. Urooj, K. Byrappa, et al., A review on medicinal plants evaluated for anti-diabetic potential in clinical trials: present status and future perspective, J. Herb. Med. vol. 28 (2021), 100436.
[13] A.M. Dirir, M. Daou, A.F. Yousef, L.F. Yousef, et al., A review of alpha-glucosidase inhibitors from plants as potential candidates for the treatment of type-2 diabetes, Phytochem. Rev. vol. 21 (4) (2022) 1049–1079.
[14] W.K. Balwan, N. Saba, J.I. Zargar, et al., Burden of diabetes and role of medicinal plants in its treatment, Saudi J. Med. Pharm. Sci. vol. 8 (7) (2022) 355–361.
[15] P. Máximo, L.M. Ferreira, P.S. Branco, A. Lourenço, et al., Invasive plants: turning enemies into value, Molecules vol. 25 (15) (2020) 3529.
[16] S. Naila, M. Ibrar, et al., Pharmacological studies of Oxalis ligh-caprae L. Pharmacogn. J. vol. 10 (4) (2018).
[17] S. Gonçalves, E. Moreira, P.B. Andrade, P. Valentão, A. Romano, et al., Effect of in vitro gastrointestinal digestion on the total phenolic contents and antioxidant activity of wild Mediterranean edible plant extracts, Eur. Food Res. Technol. vol. 245 (3) (2019) 753–762.
[18] M.C. Gaspar, et al., Polyphenolic characterisation and bioactivity of an Oxalis pes-caprae L. leaf extract, Nat. Prod. Res. vol. 32 (6) (2018) 732–738.
[19] A. Halouani, et al., Assessment of the phenolic contents, antioxidant and antimicrobial activities of Oxalis pes-caprae, Biomed. J. Sci. Tech. Res. vol. 38 (2) (2021) 30115–30124.
[20] A. Aboukhalaf, et al., Traditional knowledge and use of wild edible plants in Sidi Bennour region (Central Morocco), Ethnobot. Res. Appl. vol. 23 (11) (2022) 1–18.
[21] I. Kabach, R. Mrid, N. Bouchmaa, Y. Bouargalne, A. Nhiri, et al., Phytochemical screening, antioxidant and cytotoxic activities of M. vulgare, Int. J. Pharm. Res. vol. 11 (2019) 338–345.
[22] N. Bouchmaa, et al., Beta vulgaris subsp. maritima: a valuable food with high added health benefits, Appl. Sci. vol. 12 (4) (2022) 1866.
[23] R. Ben Mrid, et al., Phytochemical characterization, antioxidant and in vitro cytotoxic activity evaluation of Juniperus oxycedrus Subsp. oxycedrus needles and berries, Molecules vol. 24 (3) (2019) 502.
[24] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, et al., Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Radic. Biol. Med. vol. 26 (9–10) (1999) 1231–1237.
[25] T.C. Dinis, V.M. Madeira, L.M. Almeida, et al., Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers, Arch. Biochem. Biophys. vol. 315 (1) (1994) 161–169.
[26] M. Oyaizu, Studies on products of browning reactions: antioxidative activities of product of browning reaction prepared from glucosamine, Jpn. J. Nutr. Diet. (1986) 307–315.
[27] R.B. Mrid, et al., Dittrichia viscosa L. leaves: a valuable source of bioactive compounds with multiple pharmacological effects, Molecules vol. 27 (7) (2022) 2108.
[28] O. O. Guideline, 425: acute oral toxicity—up-and-down procedure, OECD Guidel. Test. Chem. vol. 2 (2001) 12–16.
[29] T. Szkudelski, The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas, Physiol. Res. vol. 50 (6) (2001) 537–546.
[30] S. Rahman, G. Jan, F.G. Jan, H.U. Rahim, et al., Phytochemical screening and antidiabetic, antihyperlipidemic, and antioxidant effects of Leptopus cordifolius Decne. in diabetic mice, Front. Pharmacol. vol. 12 (2021), 643242.
[31] N.M. Alema, G. Periasamy, G.G. Sibhat, G.H. Tekulu, M.G. Hiben, et al., Antidiabetic activity of extracts of Terminalia brownii Fresen. Stem bark in mice, J. Exp. Pharmacol. vol. 12 (2020) 61.
[32] E.J. Akpan, J.E. Okokon, E. Offong, et al., Antidiabetic and hypolipidemic activities of ethanolic leaf extract and fractions of Melanthera scandens, Asian Pac. J. Trop. Biomed. vol. 2 (7) (2012) 523–527.
[33] M. Li, et al., Treatment of obese diabetic mice with a heme oxygenase inducer reduces visceral and subcutaneous adiposity, increases adiponectin levels, and improves insulin sensitivity and glucose tolerance, Diabetes vol. 57 (6) (2008) 1526–1535.
[34] H. Aebi, Catalase. Methods of Enzymatic Analysis, Elsevier, 1974, pp. 673–684.
[35] N. Bouchmaa, et al., Cytotoxicity of new pyridazin-3 (2H)-one derivatives orchestrating oxidative stress in human triple-negative breast cancer (MDA-MB-468), Arch. Pharm. vol. 351 (12) (2018) 1800128.
[36] H. Ohkawa, N. Ohishi, K. Yagi, et al., Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal. Biochem. vol. 95 (2) (1979) 351–358.
[37] N. Brandstrup, Determination of hexokinase in tissue, J. Gerontol. vol. 12 (1957) 166–171.
[38] C.I. Pogson, R.M. Denton, et al., Effect of alloxan diabetes, starvation and refeeding on glycolytic kinase activities in rat epididymal adipose tissue, Nature vol. 216 (5111) (1967) 156–157.

[39] H.A. Ells, H.N. Kirkman, et al., A colorimetric method for assay of erythrocytic glucose-6-phosphate dehydrogenase, Proc. Soc. Exp. Biol. Med. vol. 106 (3) (1961) 607–609.
[40] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. vol. 72 (1–2) (1976) 248–254.
[41] R.K. Singla, et al.. Natural Polyphenols: Chemical Classification, Definition of Classes, Subcategories, and Structures, Oxford University Press, 2019, pp. 1397–1400.
[42] P. Tuladhar, S. Sasidharan, P. Saudagar, et al., Role of phenols and polyphenols in plant defense response to biotic and abiotic stresses. Biocontrol Agents and Secondary Metabolites, Elsevier, 2021, pp. 419–441.
[43] L. Barros, P. Morales, A.M. Carvalho, I.C. Ferreira, et al., Antioxidant potential of wild plant foods. Mediterranean Wild Edible Plants, Springer, 2016, pp. 209–232.
[44] F. Gul, et al., Phytochemistry, biological activities and in silico molecular docking studies of Oxalis pes-caprae L. compounds against SARS-CoV-2, J. King Saud Univ. Sci. vol. 34 (6) (2022), 102136.
[45] S. Mukherjee, et al., Oxalis corniculata (Oxalidaceae) leaf extract exerts in vitro antimicrobial and in vivo anticolonizing activities against Shigella dysenteriae 1 (NT4907) and Shigella flexneri 2a (2457T) in induced diarrhea in suckling mice, J. Med. Food vol. 16 (9) (2013) 801–809.
[46] X. Liao, H. Xu, P. Feng, Y. Wang, J. Huang, et al., Evaluation of environment on polyphenols and flavonoids in oxalis corymbosa extracts as a potential source of antioxidants, IOP Conf. Ser. Earth Environ. Sci. vol. 170 (5) (2018), 052034.
[47] L.H. Madkour, Reactive Oxygen Species (ROS), Nanoparticles, and Endoplasmic reticulum (er) Stress-induced Cell Death Mechanisms, Academic Press, 2020.
[48] A. Aktumsek, G. Zengin, G.O. Guler, Y.S. Cakmak, A. Duran, et al., Antioxidant potentials and anticholinesterase activities of methanolic and aqueous extracts of three endemic Centaurea L. species, Food Chem. Toxicol. vol. 55 (2013) 290–296.
[49] I. Güçlütürk, A. Detsi, E.K. Weiss, E. Ioannou, V. Roussis, P. Kefalas, et al., Evaluation of anti-oxidant activity and identification of major polyphenolics of the invasive weed Oxalis pes-caprae, Phytochem. Anal. vol. 23 (6) (2012) 642–646.
[50] H. Šircelj, M. Mikulič-Petkovšek, F. Batič, et al., Antioxidants in spring leaves of Oxalis acetosella L. Food Chem. vol. 123 (2) (2010) 351–357.
[51] U. Asmat, K. Abad, K. Ismail, et al., Diabetes mellitus and oxidative stress—a concise review, Saudi Pharm. J. vol. 24 (5) (2016) 547–553.
[52] D.D. Tibuhwa, Antioxidant potentialities and antiradical activities of Oxalis corniculata Linn from Tanzania, J. Appl. Biosci. vol. 116 (2017) 11590–11600.
[53] F. Giacco, M. Brownlee, et al., Oxidative stress and diabetic complications, Circ. Res. vol. 107 (9) (2010) 1058–1070.
[54] O.O. Oguntibeju, Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links, Int. J. Physiol. Pathophysiol. Pharmacol. vol. 11 (3) (2019) 45.
[55] J. Lim, M.G. Ferruzzi, B.R. Hamaker, et al., Structural requirements of flavonoids for the selective inhibition of α-amylase versus α-glucosidase, Food Chem. vol. 370 (2022), 130981.
[56] L.K. Williams, C. Li, S.G. Withers, G.D. Brayer, et al., Order and disorder: differential structural impacts of myricetin and ethyl caffeate on human amylase, an antidiabetic target, J. Med. Chem. vol. 55 (22) (2012) 10177–10186.
[57] J. Lim, X. Zhang, M.G. Ferruzzi, B.R. Hamaker, et al., Starch digested product analysis by HPAEC reveals structural specificity of flavonoids in the inhibition of mammalian α-amylase and α-glucosidases, Food Chem. vol. 288 (2019) 413–421.
[58] N. Rabbani, A. Ashour, P.J. Thornalley, et al., Mass spectrometric determination of early and advanced glycation in biology, Glycoconj. J. vol. 33 (4) (2016) 553–568.
[59] W.-J. Yeh, S.-M. Hsia, W.-H. Lee, C.-H. Wu, et al., Polyphenols with antiglycation activity and mechanisms of action: a review of recent findings, J. Food Drug Anal. vol. 25 (1) (2017) 84–92.
[60] H. Vlassara, J. Uribarri, et al., Advanced glycation end products (AGE) and diabetes: cause, effect, or both? Curr. Diabetes Rep. vol. 14 (1) (2014) 1–10.
[61] A. Kükürt, V. Gelen, Ö.F. Başer, H.A. Deveci, M. Karapehlivan, et al., Thiols: role in oxidative stress-related disorders, Accent. Lipid Peroxid. (2021) 27.
[62] J. Zeng, M.J. Davies, et al., Protein and low molecular mass thiols as targets and inhibitors of glycation reactions, Chem. Res. Toxicol. vol. 19 (12) (2006) 1668–1676.
[63] Z. Cai, L.-J. Yan, et al., Protein oxidative modifications: beneficial roles in disease and health, J. Biochem. Pharmacol. Res. vol. 1 (1) (2013) 15.
[64] S.F. Yan, R. Ramasamy, A.M. Schmidt, et al., Mechanisms of disease: advanced glycation end-products and their receptor in inflammation and diabetes complications, Nat. Clin. Pract. Endocrinol. Metab. (2008) 285–293.
[65] N. Pathomthongtaweechai, S. Chutipongtanate, et al., AGE/RAGE signaling-mediated endoplasmic reticulum stress and future prospects in non-coding RNA therapeutics for diabetic nephropathy, Biomed. Pharmacother. vol. 131 (2020), 110655.
[66] Q. Song, J. Liu, L. Dong, X. Wang, X. Zhang, et al., Novel advances in inhibiting advanced glycation end product formation using natural compounds, Biomed. Pharmacother. vol. 140 (2021), 111750.
[67] Q. Zhou, K.-W. Cheng, J. Xiao, M. Wang, et al., The multifunctional roles of flavonoids against the formation of advanced glycation end products (AGEs) and AGEs-induced harmful effects, Trends Food Sci. Technol. vol. 103 (2020) 333–347.
[68] A.M. Al-Attar, T.A. Zari, et al., Influences of crude extract of tea leaves, Camellia sinensis, on streptozotocin diabetic male albino mice, Saudi J. Biol. Sci. vol. 17 (4) (2010) 295–301.
[69] M.M. Qaid, M.M. Abdelrahman, et al., Role of insulin and other related hormones in energy metabolism—a review, Cogent Food Agric. vol. 2 (1) (2016) 1267691.
[70] I. Fridovich, Superoxide dismutases: an adaptation to a paramagnetic gas, J. Biol. Chem. vol. 264 (14) (1989) 7761–7764.
[71] M. Kantola, M. Saaranen, T. Vanha-Perttula, et al., Selenium and glutathione peroxidase in seminal plasma of men and bulls, Reproduction vol. 83 (2) (1988) 785–794.
[72] S. Benazzouk, P.I. Dobrev, Z.-E. Djazouli, V. Motyka, S. Lutts, et al., Positive impact of vermicompost leachate on salt stress resistance in tomato (Solanum lycopersicum L.) at the seedling stage: a phytohormonal approach, Plant Soil vol. 446 (1) (2020) 145–162.
[73] I.C. West, Radicals and oxidative stress in diabetes, Diabet. Med. vol. 17 (3) (2000) 171–180.
[74] F. Ito, Y. Sono, T. Ito, et al., Measurement and clinical significance of lipid peroxidation as a biomarker of oxidative stress: oxidative stress in diabetes, atherosclerosis, and chronic inflammation, Antioxidants vol. 8 (3) (2019) 72.
[75] D.A. Slatter, C.H. Bolton, A.J. Bailey, et al., The importance of lipid-derived malondialdehyde in diabetes mellitus, Diabetologia vol. 43 (5) (2000) 550–557.
[76] A. de Souza Bastos, et al., Diabetes and increased lipid peroxidation are associated with systemic inflammation even in well-controlled patients, J. Diabetes Complicat. vol. 30 (8) (2016) 1593–1599.
[77] D.J. Roberts, S. Miyamoto, et al., Hexokinase II integrates energy metabolism and cellular protection: akting on mitochondria and TORCing to autophagy, Cell Death Differ. vol. 22 (2) (2015) 248–257.
[78] R. Li, W. Wang, Y. Yang, C. Gu, et al., Exploring the role of glucose-6–phosphate dehydrogenase in cancer, Oncol. Rep. vol. 44 (6) (2020) 2325–2336.
[79] N. Schormann, K.L. Hayden, P. Lee, S. Banerjee, D. Chattopadhyay, et al., An overview of structure, function, and regulation of pyruvate kinases, Protein Sci. vol. 28 (10) (2019) 1771–1784.
[80] O. Macdonald Ighodaro, A. Mohammed Adeosun, O. Adeboye Akinloye, et al., Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies, Medicina vol. 53 (6) (2017) 365–374.
[81] M. Roden, G.I. Shulman, et al., The integrative biology of type 2 diabetes, Nature vol. 576 (7785) (2019) 51–60.
[82] G. Williamson, K. Sheedy, et al., Effects of polyphenols on insulin resistance, Nutrients vol. 12 (10) (2020) 3135.