Original Research

Antioxidant Activity and Inhibition of Carbohydrate Digestive Enzymes Activities of *Artemisia campestris* L.

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Abstract

Background: Carbohydrate digestive enzymes play a major role in the management of the postprandial hyperglycemia. A chronic hyperglycemia can lead to serious health problems due to excessive production of several reactive oxygen species. Therefore, the inhibition of carbohydrate digestive enzyme and the use of antioxidant natural product can be an important strategy to control the glycaemia level and prevent against the complication of diabetes. **Aim**: The study aims to perform a phytochemical analysis, antioxidant activity, inhibitory effect on α-amylase, α-glucosidase (*in vitro* and *in vivo*) and the intestinal glucose absorption in Wistar rats of *Artemisia campestris* aqueous extract (AcAE) and hydro-ethanolic extract (AcEE). **Results**: The test of total phenolic content, show that the AcAE has the highest quantity of polyphenol (44.65 ± 0.54 μg GAE/mg extract) compared to the AcEE (31.7 ± 0.53 μg GAE/mg extract) significantly. The amount of flavonoid and condensed tannins content in AcAE is 24.41 ± 3.57 μg QrE/mg extract, 14.31 ± 5.26 μg CE/mg respectively. The AcAE has also exhibit a great antioxidant activity in DPPH-scavenging and Ferric reducing antioxidant power assay (FRAP) compared to AcEE with an $IC_{50} = 0.355 \pm 0.057$ mg/mL and $IC_{50} = 0.269 \pm 0.025$ mg/mL. However, in a β-carotene bleaching assay the AcEE has the highest effect with an $IC_{50} = 0.319 \pm 0.097$ mg/mL. The both extract of *Artemisia campestris* L. (250 mg/kg) decreased postprandial hyperglycemia in the normal and alloxane diabetic rats in a very significant manner after starch or sucrose administration as an α-amylase and α-glucosidase substrate respectively. This result is confirmed *in vitro* by a remarkable inhibitory effect on α-amylase digestive enzymes by an $IC_{50} = 1.259 \pm 0.128$ mg/mL and $IC_{50} = 0.602 \pm 0.072$ mg/mL receptively for AcAE and AcEE. For the α-glucosidase enzyme, the both extracts significantly inhibit α-glucosidase activity compared to the control and they are almost similar to each other. Using a jejunum perfusion technique (*in situ*), *Artemisia campestris* L. decrease the intestinal D-glucose absorption activity significantly compared to the control and comparable to the Phlorizin used as a positive control by an amount of glucose absorbed equal a 6.53 ± 0.57, 5.34 ± 0.64 and 4.71 ± 0.24 mg/10 cm/h, for AcAE, AcEE and Phlorizin respectively. **Conclusions**: These results showed that the *Artemisia campestris* L. has highest phenolic content, antioxidant activity and demonstrated a postprandial anti-hyperglycemic effect *via* the inhibiting of the carbohydrate digestive enzyme (α-amylase and α-glucosidase) and the intestinal glucose absorption.

Keywords: α-amylase; α-glucosidase; *Artemisia campestris*; anti-hyperglycemic

1. Introduction

Diabetes mellitus is one of the oldest diseases known to mankind; the ancient Egyptians have described it for 3000 years [1]. Clinically diabetes mellitus characterized by a metabolic disorder caused a chronic hyperglycemia accompanied by a more or less significant alteration in the metabolism of carbohydrates, lipids and proteins [2]. Carbohydrate digestive enzymes play a major role in the management of intestinal glucose absorption and the postprandial hyperglycemia. This opens up a therapeutic approach based on inhibition of the digestive enzymes such as pan-

creatic α-amylase and intestinal α-glucosidase, to reduce the hydrolyzes of carbohydrate into a monosaccharide absorbed by the intestine and consequently decrease the hyperglycemia level [3]. Currently, multiple synthetic drugs are used as α-amylase and α-glucosidase inhibitors in order to manage diabetes, like Acarbose, Voglibose, and Miglitol [4]. Although, the long-term utilization of these drugs could lead to various side effects [5]. As a result, additional research is needed to uncover natural compounds extracted from medicinal plants that have the potential to suppress the carbohydrate digestive enzyme with fewer side

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effects. Medicinal plants represent the classical therapeutic source in developing countries [6]. In addition, plant species belonging to the Artemisia genus are utilized in worldwide ethnomedical practices due to their anti-diabetic characteristics [7]. *Artemisia compestris L.* is one of the Artemisia species that has historically been used to treat digestive tract disorders [8] such as gastric ulcer [9], diarrhea [10], antispasmodic [11], obesity, and also as an antidiabetic agent [12]. Different pharmacological studies showed that *Artemisia campestris* L. has antibacterial [13], antitumor [14], anti-inflammatory [15], antiplatelet [16], antispasmodic [17], antidiarrheal [18], and anti-ulcer activities [19]. Belgacem *et al.* [20] reported that *Artemisia campestris* L. has a hypoglycemic impact, however no information was provided on the mechanism of action responsible for this effect. Chronic hyperglycemia induces the production of extreme amounts of reactive oxygen species in the tissues and this progression can lead to sever organ damage. As a result, research into anti-diabetic substances or extracts with antioxidant properties is essential for the treatment of diabetes mellitus [21].

For this reason, we investigate the quantitative phytochemical analysis and the antioxidant activity of *Artemisia campestris* aqueous extract (AcAE) and hydro-ethanolic extract (AcEE) in our study. Moreover, for the first time we study the effect of AcAE and AcEE on α-amylase and α-glucosidase *in vitro* and *in vivo* and their intestinal glucose absorption in Wistar rats. In order to determine the mechanisms of action that explained the anti-diabetic activity of *Artemisia campestris* L.

2. Materials and Methods

2.1 Chemicals

The following reagents were purchased from SigmaAldrich: The starch and the sucrose powders, the α-glucosidase enzyme, the α-amylase enzyme, dinitrosalicylic acid, Acarbose, phlorizin dehydrate, D-glucose anhydrous, potassium chloride (KCl), magnesium chloride-6-hydrate ($MgCl_2 \cdot 6H_2O$), sodium chloride (NaCl), folinciocalteu, gallic acid, ascorbic acid, quercetin, DPPH, aluminum chloride ($AlCl_3$), potassium ferricyanide [$K_3Fe(CN)_6$], methanol, ethanol, chloroform, and ferric chloride ($FeCl_3$), Trichloroacetic acid (TCA). Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) purchased from ScharlauChemie S.A., Spain. Sodium hydrogen carbonate ($NaHCO_3$) were purchased from Farco Chemical Supplies, (Puerto Rico). Sodium phosphate monobasic 2-hydrate $NaH_2PO_4 \cdot 2H_2O$ purchased from Panreac, Spain. Alloxan mono-hydrate was purchased from ACROS Organics. Pentobarbital obtained from CEVA Santé Animale, France. Ether obtained from Somaprol, Casablanca, Morocco.

2.2 Plant Material

The aerial part of *Artemisia campestris* L.was provided by local herboristery and the plant was identified by Pr. Elachouri Mostafa from the department of Biology. The voucher specimen HUMPOM-151 was kept in the herbarium of faculty of sciences, Mohamed First University Oujda (Morocco).

• Aqueous extract

According to the traditional use of *Artemisia campestris*, the aqueous plant extract (AcAE) was prepared by infusion of 30 g of the aerial part in 300 mL distilled water for 30 min. The aqueous extract was obtained after filtration and evaporation to dryness in vacuo (yield: 19%). The drug extract ratio for the AcAE is 30:5.8. The extract was stored at –20 °C until use.

• Hydtro Ethanolic extract

In the dark at room temperature, 25 g of sample was soaked in 250 mL of 50% aqueous-ethanolic solution for 24 hours. The mixture was filtrated and the filtrate was evaporated to dryness at 50 °C to obtain the extract in yield of 14%. The drug extract ratio for the AcEE is 25:3.5. The hydro-ethanolic extract (AcEE) was kept at –20 °C until use.

2.3 Animals

Wistar rats were provided from the animal's house of the biology department of the Faculty of Sciences-Oujda, Morocco, they were placed under standard conditions (23 °C $\pm$ 2 °C and 12 h light-dark cycle), with free access to water and food. The animals are kept in their cages for one week before the day of gavage to allow acclimatization to laboratory conditions. All animals were cared for in accordance with the internationally accepted guide for the care and use of laboratory animals published by the United States National Institutes of Health [22]. The study was authorized by the Faculty of Sciences institutional review board in Oujda, Morroco (01/20-LBBEH-04 and 09/01/2020).

2.4 Diabetes Induction

Diabetes was induced according to the procedure described by Prince *et al.* [23]. The animals were fasted for about 16 h with accessibility to water. After that, all animals were injected intraperitoneally by alloxan (120 mg/kg.bw) dissolved in phosphate citrate buffer (pH = 3). One week after the administration was verified using a glucose oxidase-peroxidase method. The animals with the glycaemia higher than 1.25 g/L were included in the experiment.

2.5 Phytochemical Test of Artemisia campestris L.

2.5.1 Total Phenolic Content

The total phenol content of the extracts was determined by the Folin-Ciocalteu method [24] with some modifications. Firstly, 100 μL of each extract at a concentration of 10 mg/mL were mixed with 500 μL of the Folin reagent (10%) and at least 6 mL of distilled water. After 1 min and before 8 min, we added to the mixture 1.5 mL of Na_2CO_3 (20%). The volume was adjusted to 10 mL with distilled

water. The absorbance was determined at 760 nm. A standard curve was evaluated using gallic acid concentrations ranging from 15.62 to 500 μg/mL. The total phenol content was expressed as μg gallic acid equivalents per mg of dry weight of extract (μg GAE/mg of extract). All determinations have been carried out in triplicate.

2.5.2 Total Flavonoid Content

Total flavonoid content was assessed according to the aluminum chloride colorimetric method [25]. Briefly, 2 mL of distilled water and 100 μL of $AlCl_3$ (5%) were added to 300 μL of extract at a concentration 10 mg/mL. The volume is adjusted to 5 mL with distilled water. This solution was well mixed before being let to stand at room temperature for 30 min. The absorbance was measured at 425 nm. A series of concentration of quercetin (15.62–1000 μg/mL) was used as the standard to obtain the calibration curve. Total flavonoid content was expressed as μg quercetin equivalent per mg of dry weight of extract (μg QrE/mg extract). All measurements were performed in triplicate.

2.5.3 Total Tannin Condensed Content

The method described by Julkunen-Tiitto (1985) with slay modification is used to determine the condensed tannins content [26]. 50 μL of each extract or standard solution was mixed with 1.5 mL of vanillin (4% solubilized in methanol), and then 750 μL of concentrated HCl was added. The well-mixed solution was incubated at ambient temperature in the dark for 20 min. The absorbance was read at 500 nm. Catechin (1.95–1000 μg/mL) was used to make the standard curve and the results were expressed as μg Catechin equivalents per mg of extract dry weight (μg CE/mg extract). All determinations have been carried out in triplicate.

2.6 Antioxidant Activities

2.6.1 DPPH* Radical Scavenging Assay

The antiradical scavenging activity of sample was evaluated using a radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) [27]. The sample extract (200 μL) at range of concentration (25–1000 μg/mL, the choice of concentration is based on our previous study, which demonstrated that 1 mg/mL has a maximum *in vitro* antispasmodic effect [17]. For that, we made dilutions from a concentration of 1 mg/mL) was mixed with an 1800 μL of DPPH solution 0.004% solubilized in methanol. After 30 min, the absorbance was measured at 517 nm by the spectrophotometer. Ascorbic acid, a standard antioxidant, was used as a reference. All tests were performed in triplicate. Radical-scavenging activity of DPPH was calculated as the following percentage

$$\% \text{ Radical scavenging activity } = \frac{A0 - A1}{A0} \times 100$$

A0: Absorbance of DPPH alone

A1: Absorbance of sample mixed with DPPH solution

The result is presented as IC_{50} (The concentration of the samples inhibiting the radical scavenging activity by 50%).

2.6.2 Ferric Reducing Antioxidant Power Assay (FRAP)

The ferric ion (Fe^{3+}) reducing power assay was carried out according to the method described by Amarowicz *et al*. [28]. 2.5 mL of 0.2 M of phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide (1% *w/v*) were added to 1 mL of the samples dissolved in distilled water (AcAE or AcEE) at range of concentration (62–1000 μg/mL). The mixture obtained was then incubated at 50 °C for 20 min. After the incubation 2.5 mL of Trichloroacetic acid (10% *w/v*) was added. The mixture was centrifuged at 3000 rpm for 10 min and 2.5 mL of the supernatant was collected and mixed with distilled water (2.5 mL) and 0.5 mL of $FeCl_3$ (0.1% *w/v*). The absorbance was then measured at 700 nm against blank sample.

Ascorbic acid was used as a reference compound. All of the tests were performed in triplicate. The result is presented as IC_{50} (The concentration of the samples caused 50% of inhibition).

2.6.3 β-carotene/linoleic Acid Bleaching Assay

The antioxidant activity of AcAE and AcEE was carried out by measuring the inhibition of the oxidative degradation of β-carotene according to the protocol described by Miller (1970) [29] and modified by Bekkouch *et al*. [30]. Briefly, an emulsion of β carotene/linoleic acid was prepared by solubilizing 2 mg of β-carotene in 1000 μL of chloroform. Then 2 mg of linoleic acid and 200 mg of Tween (80) were added. To remove the chloroform, the mixture was then evaporated using a rotavapor. At the end, a volume of 100 mL of distilled water was added with vigorous stirring. In a series of test tubes were added a volume of 50 μL of samples or Butylated hydroxyanisole (BHA) at different concentrations (62–1000 μg/mL). A volume of 2550 μL of the β-carotene solution was the introduced into the tubes. Absorbance was measured at 490 nm before and after 2 hours incubation at 50 °C against a blank that contains ethanolfor the BHA. The measurements were made in triplicate.

The percentage of relative antioxidant activity after 2 hours was calculated according to the following formula

$$\text{Bleaching inhibition } \% = 100 - \left(\frac{\text{initial Absorbance } - \text{ Absorbance after 2 h}}{\text{initial Absorbance}} \times 100 \right)$$

The result is presented as IC_{50} (The concentration of the samples caused 50% of bleaching inhibition).

2.7 In Vitro Inhibition Assay for Digestive Enzymes

The α-amylase and α-glucosidase inhibition activity by AcAE and AcEE was studied according to the procedure described by Daoudi *et al*. [31] with some minor modifications. The choice of the concentration is made according to the preliminary validation tests that we have carried out.

2.7.1 In Vitro α-amylase Inhibition

The assay mixtures contained 200 μL of porcine pancreatic α-amylase enzyme solution (13 IU), 200 μL of phosphate buffer (0.02 M; pH = 6.9) and 200 μL of sample (AcAE, AcEE or acarbose) at the following concentrations (0.56, 1.12, 2.25 mg/mL). The mixtures were pre-incubated at 37 °C for 10 min. Then, 200 μL of starch (1%) dissolved in phosphate buffer was added to each tube and were incubated for 20 min at 37 °C. Then, 600 μL of 3.5-dinitrosalicylic acid (DNSA) color reagent was added to stop the enzymatic reaction. The tubes were then incubated for 8 minutes at 100 °C. After that, they were immersed in an ice-cold water bath for a few minutes. The mixture was diluted by adding 1 mL of distillate water and the absorbance was measured at 540 nm. All tests were performed in triplicate. The inhibition percentage was calculated using the below formula:

$$\text{Inhibitory activity (\%)} = \frac{\text{OD Test} - \text{OD Control}}{\text{OD Test}} \times 100$$

The concentration of the samples inhibiting the enzymatic activity of α-amylase by 50% (IC_{50}) was calculated graphically according to the function:

$$\textbf{Inhibition percentage} = \text{f (log sample concentraction)}$$

2.7.2 In Vitro α-glucosidase Inhibition

The effect of AcAE and AcEE on α-glucosidase activity was calorimetrically quantified by monitoring the glucose release from sucrose degradation. The assay mixtures contained 100 μL of sucrose (50 mM), 1000 μL of phosphate buffer (50 mM; pH = 7.5) and 100 μL of α-glucosidase enzyme solution (10 IU). Then, 20 μL of acarbose, AcAE or AcEE at two different concentrations: 328 and 656 μg/mL were added to the mixture. The same volume of distilled water was replaced in the control. Then, it was incubated at 37 °C in water bath for 25 min. After, the mixture was heating at 100 °C for 5 min in order to stop the enzymatic reaction, and the release glucose was estimated by glucose oxidase method using a commercially auto-kit (The GOD-POD is a colored glucose indicator, purchased from Biosystems. S.A Barcelona, Spain). The absorbance was measured at 500 nm. All tests were performed in triplicate. The inhibition percentage was calculated using the formula:

$$\text{Inhibitory activity (\%)} = \frac{\text{OD Control} - \text{OD Test}}{\text{OD Control}} \times 100$$

Principe of GOD-POD method: Glucose oxidase enzyme (GOD) oxidizes the specific substrate D-glucose to gluconic acid and hydrogen peroxide (H_2O_2) is liberated. Peroxidase enzyme acts on hydrogen peroxide to liberate oxygen (O). Nascent oxygen then couples with 4-aminoantipyrine and phenol to form red quinoneimine dye. The intensity of color is directly proportional to concentration of glucose in plasma. The intensity of color is measured colorimetrically at 500 nm [32].

2.8 In Vivo, Inhibition for Digestive Enzymes

To evaluate the activity of the both extracts in α-amylase and α-glucosidase *in vivo*, we followed the protocol described by Subramanian *et al*. [33]. Normal and diabetic Wistar rats weighing 150–200 g, were deprived of food 16 h before each test. The rats were randomly divided into four groups with five animals in each. The control group, the acarbose group (Positive control), the AcAE group and the AcEE group. In fact, the concentrations choice is based on the previous work that published on the same topics [34]. The different groups received respectively the distillate water (10 mL/kg), Acarbose (10 mg/kg), AcAE (250 mg/kg) and AcEE (250 mg/kg). After 30 min of solutions administration, the animals were received orally using a specific gavage tube, starch (2 g/kg) as an α-amylase substrate, or sucrose (2 g/kg) as an α-glucosidase substrate. Then, the blood glucose was estimated at different times: 0, 30, 60 and 120 min, using the glucose-peroxidase method (The GOD-POD). Moreover, the diabetic rats were treated similarly to the normal rats.

2.9 In Situ Intestinal Glucose Absorption

Intestinal glucose absorption, was evaluated using a jejunum segments perfusion technique [34]. Primarily, normal Wistar rats were deprived of food before the assay for 36 h, with free access to water. Rats were divided into 4 groupswith five animals in each: The control group received the perfusion solution (in g/L: 7.37 NaCl, 0.2 KCl, 0.065 $NaH_2PO_4 \cdot 2H_2O$, 0.213 $MgCl_2 \cdot 6 H_2O$, 0.6 $NaHCO_3$, and 1.02 $CaCl_2 \cdot 2H_2O$). The positive control group received the perfusion solution with the phlorizin (0.2 mM). The third and fourth group received the perfusion solution with the AcAE or AcEE (250 mg/kg) respectively. D-glucose (1 g/L) was added to the solutions just before the start of the experiment and the pH was kept at 7.5. The animals were anesthetized by intramuscular injection of 50 mg/kg of pentobarbital, and fixed on a homoeothermic plate (37 °C). Then, 10 cm of the jejunum segment is perfused with a solution according to the group studied, using a syringe Pump at 0.53 mL/min. After 60 min, the perfuse was collected in order to estimate the amount of glucose in final solutions using the glucose oxidase peroxidase method and the length of segments were measured in cm. The results are expressed in mg/cm/h corresponding of amount of absorbed glucose (mg) per length of the segment (cm) per

time of perfusion (60 min).

2.10 Statistical Analysis

The results were expressed as the mean $\pm$ S.E.M. Moreover, the statistical analysis was performed differently depending on the experiment:

• Two-way analysis of variance (ANOVA) for *in vivo* and *in vitro* inhibitory digestive enzymes.

• One-way followed by a post hoc Tukey test for *in situ* intestinal glucose absorption test.

• Student's *t* test to analyze the results of the phytochemical study and the IC_{50} of the antioxidant tests.

The statistical analysis was performed using a Graph-Pad Prism software, version 5.01 (San Diego, CA, USA). The difference was significant when *p* is less than 5%. The concentration of the samples inhibiting 50% of activities demonstrated (IC_{50}) was calculated graphically according to the function [Inhibition percentage = f(log sample concentraction)].

3. Results

3.1 Phytochemical Test of Artemisia campestris L.

The Table 1 shows the quantitative phytochemical analysis of the both extract from *Artemisia campestris* L. The AcAE possess a high quantity of phenolic, flavonoid content and condensed tannins by 44.65 $\pm$ 0.54 μg GAE/mg, 24.41 $\pm$ 3.75 μg QrE/mg and 14.31 $\pm$ 5.26 μg CE/mg of extract respectively. For the hydro-ethanolic extract (AcEE) the total phenolic, flavonoid content and condensed tannins is 31.7 $\pm$ 0.53 μg GAE/mg of extract, 17.31 $\pm$ 1.69 μg QrE/mg of extract and 10.65 $\pm$ 4.93 μg CE/mg of extract respectively.

Table 1. The quantitative phytochemical analysis of different extract from *Artemisia campestris* L.

	TPC μg GAE/mg	TFC μg QrE/mg	TCC μg CE/mg
AcAE	44.65 $\pm$ 0.54*	24.41 $\pm$ 3.57	14.31 $\pm$ 5.26
AcEE	31.7 $\pm$ 0.53	17.31 $\pm$ 1.69	10.65 $\pm$ 4.93

Data are expressed as mean $\pm$ SEM (n = 3). Student's *t* test to analyze the results. * *p* < 0.05, difference is significant between the two extracts.

TPC, Total phenolic content (μg GAE/mg: μg of Gallic Acid equivalent per mg of extract); TFC, Total flavonoid content (μg QrE/mg: μg of Quercetin equivalent per mg of extract); TCC, Total tannins condensed content (μg CE/mg: μg of Catechin equivalent per mg of extract).

3.2 Antioxidant Activity of Artemisia campestris L.

The antioxidant activity was investigated by three methods, the antiradical scavenging activity using DPPH radical, β-carotene bleaching assay, and the ferric reducing power activity.

The results obtained showed that the AcAE and AcEE have a dose dependent antiradical scavenging activity, increased significantly by increasing the concentration with a maximum effect in a dose 1 mg/mL for the both extract (Fig. 1). In the other hand the aqueous extract of *Artemisia campestris* have a low value of IC_{50} (0.355 $\pm$ 0.057 mg/mL) compared with AcEE (0.450 $\pm$ 0.060 mg/mL) ($p < 0.01$) that is to say the AcAE has an important antioxidant power. The IC_{50} obtained by ascorbic acid used as a positive control is 0.063 $\pm$ 0.003 mg/mL (Table 2).
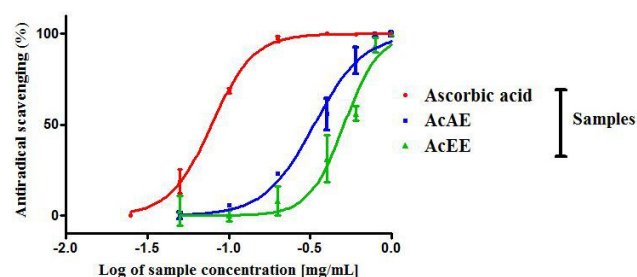

Fig. 1. Antiradical-scavenging activity against DPPH of *Artemisia campestris* aqueous extract (AcAE) and hydro-ethanolic extract (AcEE). Each value represent means $\pm$ SEM (n = 3).

Table 2. IC_{50} value of antioxidant activities of *Artemisia campestris* L extracts using three methods: DPPH-scavenging, β-carotene bleaching and FRAP.

	IC_{50} (mg/mL)		
Sample	DPPH	β-carotene bleaching	FRAP
AcAE	0.355 $\pm$ 0.057**	0.524 $\pm$ 0.011	0.269 $\pm$ 0.025*
AcEE	0.450 $\pm$ 0.060	0.319 $\pm$ 0.097	0.396 $\pm$ 0.021
Ascorbic acid	0.063 $\pm$ 0.003		0.130 $\pm$ 0.003
BHA		0.212 $\pm$ 0.043	

Student's *t*-test to analyze the results. *$p < 0.05$; **$p < 0.01$, difference is significant between the two extracts. Data are expressed as mean $\pm$ SEM (n = 3).

IC_{50} value was determined to be the effective concentrations at which affect an antioxidant activity by 50%.

The ferric reducing antioxidant power assays are represented in Fig. 2. As demonstrated by the fact that the AcAE has the largest reducing power activity when compared to the AcEE, with a dose dependent effect and a maximum at 1 mg/mL for both extracts, this is confirmed by the fact that the IC_{50} of the AcAE is 0.269 $\pm$ 0.025 mg/mL followed by the AcEE with IC_{50} value of 0.396 $\pm$ 0.021 mg/mL. The difference between the IC_{50} of the two extracts is statistically significant ($p < 0.05$). Moreover, this effect is lower than the ascorbic acid with an $IC_{50} = 0.130 \pm 0.003$ mg/mL (Table 2).
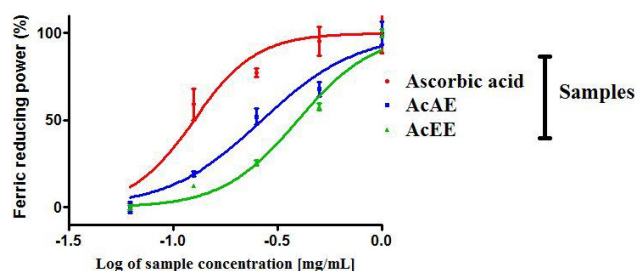
Fig. 2. Ferric reducing antioxidant power (FRAP) of *Artemisia campestris* aqueous extract (AcAE) and ethanolic extract (AcEE). Each value represent means $\pm$ SEM (n = 3).

The AcAE and AcEE inhibited the bleaching of β-carotene with a dose dependent manner with also a maximum effect at 1 mg/mL (Fig. 3). The highest activities were found by the AcEE (IC$_{50}$ = 0.319 $\pm$ 0.097 mg/mL) followed by AcAE (0.524 $\pm$ 0.011 mg/mL). The positive control (BHA) exhibits a great inhibited of β-carotene bleaching compared to the two extract of *Artemisia campestris* with IC$_{50}$ = 0.212 $\pm$ 0.043 mg/mL (Table 2).
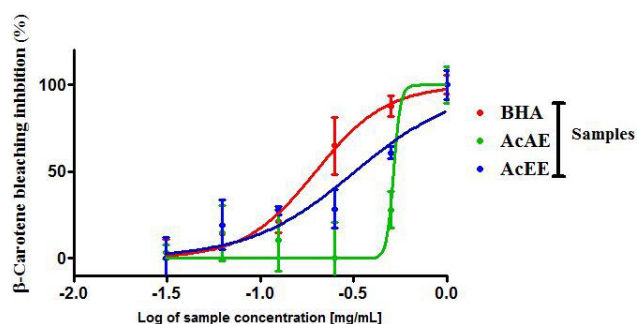
Fig. 3. The β-carotene bleaching assay of *Artemisia campestris* aqueous extract (AcAE) and hydro-ethanolic extract (AcEE). Each value represent means $\pm$ SEM (n = 3).

3.3 In Vitro Inhibition Assay for Digestive Enzymes

Artemisia campestris L. was tested for its inhibitory activity against the enzymes α-amylase and α-glycosidase. The α-amylase inhibitory potential of AcAE and AcEE exhibited a dose-dependent inhibition and very significant compared to the control. The AcEE induced the greatest activity against α-amylase with an inhibition of 32.68, 70.16, and 78.51% for 0.56, 1.12, 2.25 mg/mL compared with the AcAE that exhibited an inhibition of 18.99, 41.87, and 62.31% for 0.56, 1.12, 2.25 mg/mL respectively. The acarbose used as a reference drug induced an inhibition of 51.34, 68.30, and 79.43% for the same dose (Fig. 4). According to these results, AcEE has an effect comparable to that of acarbose, especially in high doses, which is confirmed by the IC$_{50}$ of the extract which equal to 0.602 $\pm$ 0.072 mg/mL, for acarbose is 0.417 $\pm$ 0.014 mg/mL (Table 3).
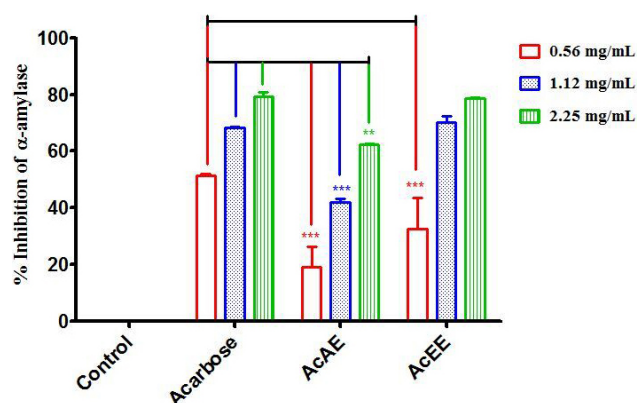

Fig. 4. *In vitro* inhibition of the enzyme α-amylase by *Artemisia campestris* aqueous extract (AcAE), hydro-ethanolic extract (AcEE) and acarbose (reference drug). The difference between the groups was calculated with a two-way analysis of variance (ANOVA). Each value represent means $\pm$ SEM. All dose tests of each groups are very significant compared to control group. ** $p < 0.01$, *** $p < 0.001$. Compared to acarbose group (n = 3).

Table 3. IC$_{50}$ Value of inhibition of α-amylase by *Artemisia campestris* aqueous extract (AcAE), hydro-ethanolic extract (AcEE) and acarbose (reference drug).

Sample	IC$_{50}$ (mg/mL)
AcAE	1.259 $\pm$ 0.128
AcEE	0.602 $\pm$ 0.072
Acarbose	0.417 $\pm$ 0.014

Each value represent means $\pm$ SEM (n = 3).
IC$_{50}$: concentration of sample that giving half-maximal inhibitory response.

In terms of the effect of AcAE and AcEE on α-glucosidase inhibition, both extracts inhibit significantly α-glucosidase activity compared to the control and they are almost similar to each other. The most active concentration for the both extract of *Artemisia campestris* L. is 656 μg/mL by an enzyme inhibition of 81.76% for AcAE and 81.18% for the AcEE (Fig. 5).

3.4 In Vivo Inhibition Assay for a Digestive Enzyme

3.4.1 In Vivo α-amylase Inhibitory Effect

The results showed that the AcAE and AcAE (250 mg/kg) decreased postprandial glycaemia in the normal rats at time 30 and 60 min in a very significant manner ($p < 0.001$) compared to the control which increased the glycaemia level from 0.9 g/L up to 1.5 g/L after starch administration. However, the blood glucose level at time 120 min showed no significant difference between the both extracts group and the control group. Concerning the group received the acarbose (10 mg/kg) show similar effect compared with the AcAE and AcEE at time 30 and 60 min (Fig. 6A). In the control group of alloxane-diabetic rats (Fig. 6B), the
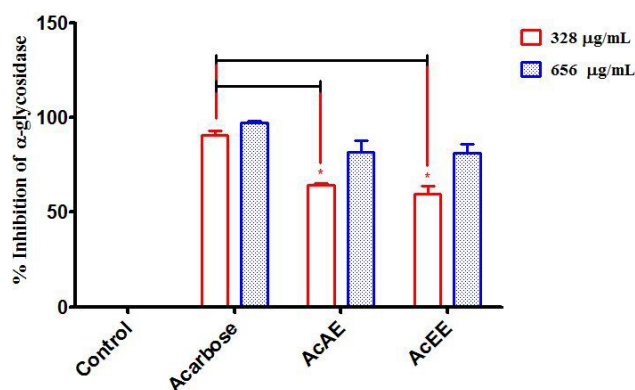
Fig. 5. *In vitro* **inhibition of the enzyme α-glycosidase by *Artemisia campestris* L. aqueous extract (AcAE), hydroethanolic extract (AcEE) and acarbose (reference drug).** The difference between the groups was calculated with a two-way analysis of variance (ANOVA). Each value represent means ± SEM. All dose tests of each group are very significant compared to control group. * $p < 0.05$. Compared to acarbose (n = 3).

glycaemia increased from 3.67 g/L to 4.66 g/L at 30 min. This glycaemia decreases significantly in presence of the both extract ($p < 0.01$) and very significantly in the acarbose group ($p < 0.01$).

3.4.2 *In Vivo* α-glucosidase Inhibitory Effect

The results of the effect of the extracts of *Artemisia campestris* L. on the inhibition of α-glucosidase *in vivo* in normal rats was illustrated in Fig. 7A. After sucrose administration in the control group, the glycaemia increased from 0.94 g/L to 1.56 g/L at 30 min and continued to rise until 1.59 g/L. In the presence of AcAE and AcEE (250 mg/kg), the blood sugar decrease significantly ($p < 0.001$) at 30 min compared with the control group. At 60 and 120 min, the glycaemia level continues to decrease very significantly ($p < 0.001$) compared to the control group and until more than acarbose group. However, in alloxan diabetic rats, the postprandial glucose concentrations increase in the control group from 3.45 g/L to 4.086 g/L after 30 min. On the other hand, in the group treated with acarbose, blood sugar levels were reduced by 3.21 g/L to 2.61 g/L at 30 min, the same remark observed in the AcAE and AcEE groups (Fig. 7B).

3.4.3 *In Situ* Intestinal Absorption

The results of the intestinal glucose absorption study show that the amount of glucose absorbed in the control group is 12.18 ± 0.61 mg/10 cm/h. This amount decreases significantly in presence of AcAE ($p < 0.001$) and AcEE ($p < 0.001$) with amount of 6.53 ± 0.57 and 5.34 ± 0.64 mg/10 cm/h respectively. These results are comparable with the result obtained in phlorizin group with an amount of glucose-absorbed equal a 4.71 ± 0.24 mg/10 cm/h (Fig. 8).

4. Discussion

The purpose of this study is to assess the inhibitory effect of *Artemisia campestris* L. on carbohydrate Digestive enzymes, particularly against α-amylase and -glucosidase, as well as their intestinal glucose absorption activity. Additionally, we investigated phytochemical analysis of AcAE and AcEE and their antioxidant capacity.

Starch is normally the main source of digestible carbohydrates in human alimentation, and it is the major source of glucose that appears at fairly high concentrations in the blood circulation following intestinal digestion, α-amylase and α-glucosidase are two primary enzymes involved in carbohydrate digestion. The first step in digestion of starch is catalyzed by α-amylase [35], that present in salivary and pancreatic secretions and catalyzing the hydrolysis of α-(1,4)-glycosidic linkages in starch, forming an oligosaccharides or disaccharides. The second step α-glucosidase that is fond on luminal surface of enterocytes, hydrolyzes non-reducing α-(1,4) bond of oligosaccharides or disaccharides and releases glucose molecules [36,37]. The AcAE and AcEE (250 mg/kg) inhibit the hyperglycemia level due to administration of starch and sucrose as an α-amylase and α-glucosidase substrates respectively in normal and diabetic rats, in a short time significantly compared to the control and exhibit a similar activity as acarbose (10 mg/kg). In other words, our extracts have an inhibitory effect against α-amylase and α-glucosidase *in vivo*, which confirmed by the fact that hydrolysis effect of these enzymes inhibited *in vitro* significantly by AcAE and AcEE. In orally induced hyperglycemia model, the n-butanol Fraction from Tunisian *Artemisia campestris* L. at the highest doses of 550 and 400 mg/kg.bw significantly reduced the postprandial hyperglycemic peak compared to control [20]. Two other Moroccan Asteraceae species (*Bubonium imbricatum* Cav. and *Cladanthus arabicus* (L.)) had an enzyme inhibitory activity against various enzymes such as α-glucosidase [38]. Several extracts from the *Artemisia* genus of the Asteraceae family shown inhibitory effect against the key enzymes involved in carbohydrate metabolism, such as α-amylase (IC_{50} = 150.24–384.14 μg/mL) and α-glucosidase (IC_{50} = 214.42–754.12 μg/mL) [7].

The results of the phytochemical analysis of *Artemisia campestris* L. demonstrated that the AcAE and AcEE contain an important quantity of polyphenol, flavonoid and tannin. Similar results were obtained in a previous study [39]. Flavonoids such luteolin, quercetin, kaempferol, apigenin, myricetin, hesperidin, naringenin and rutin are reported to be abundant in the species *Artemisia campestris* L. Additionally, this plant contains the phenolic acids as well as chlorogenic acid, 3,4-dicaffeoylquinic acid (chlorogenic acid A), 3,5-dicaffeoylquinic acid (chlorogenic acid B), 4,5-dicaffeoylquinic acid (chlorogenic acid C) [40]. That already performed and confirmed by our previous work [41]. This may lead to establish a relationship between these phytoconstituents and the possible effect on the inhibitory of
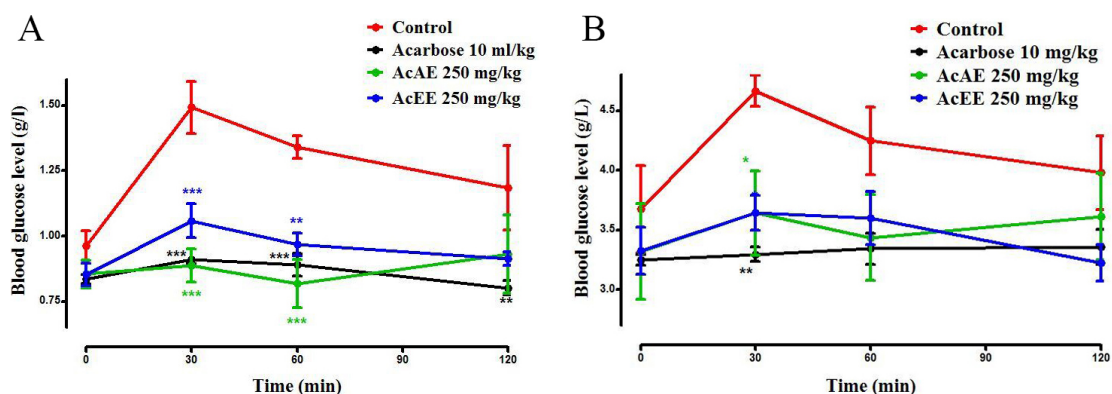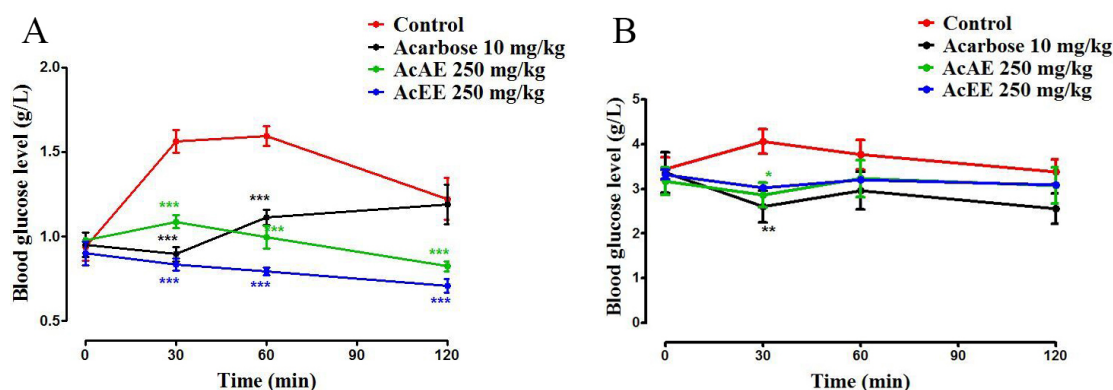
Fig. 6. Effect of *Artemisia campestris* L. Aqueous extract (AcAE) and hydro-ethanolic extract (AcEE) on blood glucose level in normal (A) and diabetic rat (B) after consumption of starch as an α-amylase substrate. Data represent mean $\pm$ SEM (n = 5). The difference between the groups was calculated with a two-way analysis of variance (ANOVA). Each value represent means $\pm$ SEM. $* p < 0.05$, $** p < 0.01$, and $*** p < 0.001$ compared to the control group.

Fig. 7. Effect of *Artemisia campestris* L. Aqueous extract (AcAE) and hydro-ethanolic extract (AcEE) on blood glucose level in normal (A) and diabetic rat (B) after consumption of sucrose as an α-glucosidase substrate. Data represent mean $\pm$ SEM (n = 5). The difference between the groups was calculated with a two-way analysis of variance (ANOVA). Each value represent means $\pm$ SEM. $* p < 0.05$, $** p < 0.01$, and $*** p < 0.001$ compared to the control group.
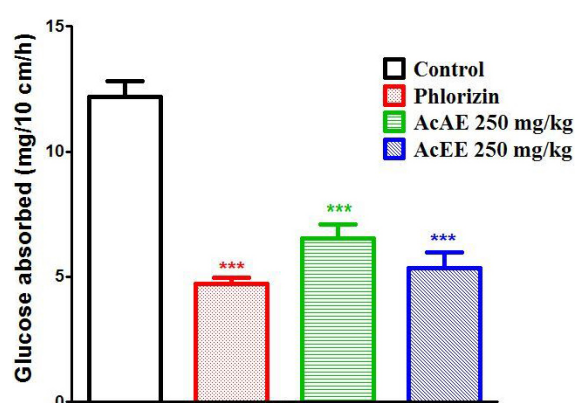

Fig. 8. Effect of AcAE and AcEE on intestinal glucose absorption in Wistar rat. Data represent mean $\pm$ SEM (n = 5). The difference between the groups was calculated with a one-way analysis of variance (ANOVA). Each value represent means $\pm$ SEM $*** p < 0.001$ compared to the control group.

carbohydrate digestive enzymes. That confirmed by the fact that the chlorogenic acid was the key to the biological response of *Artemisia* extracts by the direct relationship between it and their enzyme inhibitory activity [7]. Furthermore, chlorogenic acid from coffee has an inhibitory effect against pancreatic α-amylase [42]. In addition, naturally flavonoid especially four flavonols (quercetin, quercetagetin, myricetin, and fisetin) and three flavones (luteolin, eupafolin, and scutellarein) exhibited IC_{50} values less than 100 μM against human salivary α-amylase activity. This inhibitory effect depends on hydrogen bonds between the hydroxyl groups of the polyphenol ligands and the catalytic residues of the binding site [43]. Miao *et al.* [44] had mentioned in their work that six types of catechins has an inhibitory effect against α-amylase enzyme. This effect due to the higher binding affinity with enzyme by the interacting with the catalytic residues of the active site forming a phenols-protein complex, including hydroxyl on the 3-position or 5-position of A-C rings. In porcine pancreatic α-

amylase, another flavonoid has a more important inhibitory effect such as quercetin, luteolin and myricetin with an IC_{50} less than 500 μM. This inhibitory effect is caused by the following structures: the 2, 3-double bond, 5-OH, the linkage of the B ring at the 3 position, and the hydroxyl substitution on the B ring [45]. Another new study described that quercetin interacts with three amino acids (Arg195, Glu233, and Gln63) to catalyze the active site of α-amylase by non-covalent interaction. Therefore, molecular modeling was investigated that the flavonoid-enzyme complexes exhibiting the competitive inhibition mechanism [46]. Yeast α-glucosidase and rat small intestinal α-glucosidase was highly inhibited by several flavonoids such as flavanols, isoflavones and the anthocyanidins with an IC_{50} value less than 13 μM. This inhibitory effect wasenhanced by the following structures: the unsaturated C ring, 3-OH, 4-CO, the linkage of the B ring at the 3 position, and the hydroxyl substitution on the B ring [45]. Tannins inhibit the activity of many different enzymes by the explore tannin/protein interactions. This type of secondary metabolite frequently interferes with digestive enzymes such as trypsin, α-amylase and β-glucosidase by binding the either the enzyme or the substrate or to both [47]. Moreover, various studies showed a potent inhibitory effect of tannin on both α-amylase and α-glucosidase activities *in vitro* [48,49]. The both extracts exhibited an inhibition of intestinal glucose absorption significantly and it was similar to the phlorizin used as reference drug, and it is isolated from genus Malus. This natural drug blocked the intestinal glucose absorption through inhibition of the sodium-glucose transporter (SGLT) which reduced the glycaemia level [50]. That is to say, the effect of the plant mayact via the same pathway, but also can act via the inhibition of $GLUT_2$, or both at the same time.

Free radicals are the principal agents involved in several diseases, such as diabetes mellitus, and the prolonged hyperglycemia can provoke the production of too much reactive oxygen species (ROS) in tissues. The high contents of antioxidants compounds decrease the negative effect of free radicals and protecting against the damage induced by ROS [51,52]. However, the antioxidants effect exhibited by AcAE and AcEE in various *in vitro* assay (DPPH, FRAP and β-carotene) can support the pharmacological activities showed. These results are demonstrated by various studies and explained by the presence of artemisinin obtained in *Artemisia campestris* L. with a remarkable antioxidant capacity [38,53].

5. Conclusions

Artemisia campestris L. has highest phenolic content, antioxidant activity against DPPH, β-carotene and FRAP. Moreover, AcAE and AcEE demonstrated a postprandial anti-hyperglycemic effect *via* the inhibiting of the carbohydrate digestive enzyme (α-amylase and α-glucosidase) and the intestinal glucose absorption. These results explain the use of this plant as an antidiabetic agent and support the pharmacological basis behind this use.

Abbreviations

AcAE, *Artemisia campestris* aqueous extract; AcEE, *Artemisia campestris* hydro-ethanolic extract; FRAP, Ferric reducing antioxidant power; DPPH, 2,2-diphenyl-1-picrylhydrazyl; BHA, Butylatedhydroxyanisole; TPC, Total phenolic content; TFC, Total flavonoid content; TCC, Total tannins condensed content.

Author Contributions

MM and NED and MB and MA (Mohammed Aziz) designed the research study. MM and NED performed the experiments. OA and MA (Mohamed Addi) provided help and advice on animal experiment. HM and AZ analyzed the data. MM and NED wrote the manuscript. J-TC, CH, MA (Mohamed Addi) and MA (Mohammed Aziz) review and editing manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

All animals were cared for in accordance with the internationally accepted guide for the care and use of laboratory animals published by the United States National Institutes of Health. The study was authorized by the Faculty of Sciences institutional review board in Oujda, Morocco (01/20-LBBEH-04 and 09/01/2020).

Conflict of Interest

The authors declare no conflict of interest. JT Chen is serving as one of the Guest editors of this journal. We declare that JT Chen had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Federica Finetti.

References

[1] Ahmed AM. History of diabetes. Saudi Medical Journal. 2002; 23: 373–378.

[2] Baynest HW. Classification, Pathophysiology, Diagnosis and Management of Diabetes Mellitus. Diabetes & Metabolism Journal. 2015; 6: 1–9.

[3] Tundis R, Loizzo MR, Menichini F. Natural Products as α-Amylase and α-Glucosidase Inhibitors and their Hypoglycaemic Potential in the Treatment of Diabetes: An Update. Mini-Reviews in Medicinal Chemistry. 2010; 10: 315–331.

[4] Derosa G, Maffioli P. α-Glucosidase inhibitors and their use in clinical practice. Archives of Medical Science. 2012; 8: 899–906.

[5] Aoki K, Muraoka T, Ito Y, Togashi Y, Terauchi Y. Comparison of Adverse Gastrointestinal Effects of Acarbose and Miglitol in Healthy Men: a Crossover Study. Internal Medicine. 2010; 49: 1085–1087.

[6] WHO. Organización Mundial de la Salud Ginebra Estrategia de la OMS sobre medicina tradicional 2002–2005. WHO publisher. Available at: https://apps.who.int/iris/handle/10665/95008 (Accessed: 1 September 2021).

[7] Olennikov DN, Chirikova NK, Kashchenko NI, Nikolaev VM, Kim SW, Vennos C. Bioactive phenolics of thegenus Artemisia (Asteraceae): HPLC-DAD-ESI-TQ-MS/MS profile of the Siberian species and their inhibitory potential against α-amylase and α-glucosidase. Frontiers in Pharmacology. 2018; 9: 1–27.

[8] Jamila F, Mostafa E. Ethnobotanical survey of medicinal plants used by people in Oriental Morocco to manage various ailments. Journal of Ethnopharmacology. 2014; 154: 76–87.

[9] Leporatti ML, Ghedira K. Comparative analysis of medicinal plants used in traditional medicine in Italy and Tunisia. Journal of Ethnobiology and Ethnomedicine. 2009; 5: 31.

[10] Sassi AB, Harzallah-Skhiri F, Aouni M. Investigation of some Medicinal Plants from Tunisia for Antimicrobial Activities. Pharmaceutical Biology. 2007; 45: 421–428.

[11] Ouelbani R, Bensari S, Mouas TN, Khelifi D. Ethnobotanical investigations on plants used in folk medicine in the regions of Constantine and Mila (North-East of Algeria). Journal of Ethnopharmacology. 2016; 194: 196–218.

[12] Bnouham M, Mekhfi H, Legssyer A, Ziyyat A. Medicinal plants used in the treatment of diabetes in Morocco. International Journal of Diabetes and Metabolism. 2002; 10: 33–50.

[13] Naili MB, Alghazeer RO, Saleh NA, Al-Najjar AY. Evaluation of antibacterial and antioxidant activities of *Artemisia campestris* (Astraceae) and Ziziphus lotus (Rhamnacea). Arabian Journal of Chemistry. 2010; 3: 79–84.

[14] Akrout A, Gonzalez LA, El Jani H, Madrid PC. Antioxidant and antitumoractivities of *Artemisia campestris* and Thymelaea hirsuta fromsouthernTunisia. Food and ChemicalToxicology. 2011; 49: 342–347.

[15] Ghlissi Z, Sayari N, Kallel R, Bougatef A, Sahnoun Z. Antioxidant, antibacterial, anti-inflammatory and wound healing effects of *Artemisia campestris* aqueous extract in rat. Biomedicine & Pharmacotherapy. 2016; 84: 115–122.

[16] Dib I, Fauconnier M, Sindic M, Belmekki F, Assaidi A, Berrabah M, *et al*. Chemical composition, vasorelaxant, antioxidant and antiplatelet effects of essential oil of *Artemisia campestris* L. from Oriental Morocco. BMC Complementary and Alternative Medicine. 2017; 17: 1–15.

[17] Marghich M, Amrani O, Mekhfi H, Ziyyat A, Bnouham M, Aziz M. Myorelaxant and antispasmodic effect of an aqueous extract of *Artemisia campestris*L. via calcium channel blocking and anticholinergic pathways. Journal of Smooth Muscle Research. 2021; 57: 35–48.

[18] Marghich M, Amrani O, Makrane H, Aziz M. Antidiarrheal activity of aqueous extract of *Artemisia campestris*L. Subsp. glutinosa. Tropical Journal of Natural Product Research. 2021; 5: 1246–1249.

[19] Marghich M, Daoudi NE, Mamri S, Amrani O, Mekhfi H, Ziyyat A,*et al*. Antiulcer activity of Moroccan *Artemisia campestris* L. subsp. glutinosa against ethanol-induced gastric ulcer in Mice.

Arabian Journal of Medicinal and Aromatic Plants. 2022; 8: 20–39.

[20] Belgacem A, Ben Gdara N, Khemiri I, Bitri L. Exploration of hypoglycemic effect of an extract from leaves of a plant from Tunisian pharmacopeia: *Artemisia campestris* (asteraceae). African Health Sciences. 2019; 19: 2846–2853.

[21] Orhan N, Gökbulut A, DeliormanOrhan D. Antioxidant potential and carbohydrate digestive enzyme inhibitory effects of five Inula species and their major compounds. South African Journal of Botany. 2017; 111: 86–92.

[22] Guide for the Care and Use of Laboratory Animals, 8th edition National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. National Academies Press (US): Washington (DC). 2011.

[23] Prince PSM, Menon VP, Pari L. Hypoglycaemic activity of Syzigiumcumini seeds: effect on lipid peroxidation in alloxan diabetic rats. Journal of Ethnopharmacology. 1998; 61: 1–7.

[24] Singleton VL, Orthofer R, Lamuela-Ravents RM. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. Methods in Enzymology. 1974; 299: 152–177.

[25] Woisky RG, Salatino A. Analysis of propolis: some parameters and procedures for chemical quality control. Journal of Apicultural Research. 1998; 37: 99–105.

[26] Julkunen-Tiitto R. Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. Journal of Agricultural and Food Chemistry. 1985; 33: 213–217.

[27] Huang B, Ke H, He J, Ban X, Zeng H, Wang Y. Extracts of Haleniaelliptica exhibit antioxidant properties *in vitro* and *in vivo*. Food and Chemical Toxicology. 2011; 49: 185–190.

[28] Amarowicz R, Pegg RB, Rahimi-Moghaddam P, Barl B, Weil JA. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chemistry. 2004; 84: 551–562.

[29] Miller HE. A simplified method for the evaluation of antioxidants. Journal of the American Oil Chemists Society. 1971; 48: 91.

[30] Bekkouch O, Harnafi M, Touiss I, Khatib S, Harnafi H, Alem C, *et al*. *In vitro* Antioxidant and *in vivo* Lipid-Lowering Properties of *Zingiber officinale* Crude Aqueous Extract and Methanolic Fraction: A Follow-Up Study. Evidence-based Complement. Evidence-Based Complementary and Alternative Medicine. 2019; 2019: 9734390.

[31] Daoudi NE, Bouhrim M, Ouassou H, Legssyer A, Mekhfi H, Ziyyat A, *et al*. Inhibitory effect of roasted/unroasted Argania spinosa seeds oil on α-glucosidase, α-amylase and intestinal glucose absorption activities. South African Journal of Botany. 2020; 135: 413–420.

[32] Sharma SP, Anjankar AP, Kale A. Comparison of glucose levels using glucometer and GOD-POD Method in diabetic patients. International Journal of Clinical Biochemistry and Research. 2017; 4: 6–10.

[33] Subramanian R, Asmawi MZ, Sadikun A. *In vitro* α-glucosidase and α-amylase enzyme inhibitory effects of Andrographispaniculata extract and andrographolide. ActaBiochimicaPolonica. 2008; 2: 391–398.

[34] Ansari P, Azam S, Hannan JMA, Flatt PR, Abdel Wahab YHA. Anti-hyperglycaemic activity of H. rosa-sinensis leaves is partly mediated by inhibition of carbohydrate digestion and absorption, and enhancement of insulin secretion. Journal of Ethnopharmacology. 2020; 253: 112647.

[35] Butterworth PJ, Warren FJ, Ellis PR. Human α-amylase and starch digestion: an interesting marriage. Starch - StäRke. 2011; 63: 395–405.

[36] Yang C, Yen Y, Hung K, Hsu S, Lan S, Lin H. Inhibitory effects of pu-erh tea on alpha glucosidase and alpha amylase: a systemic

review. Nutrition & Diabetes. 2019; 9: 23.

[37] Brayer GD, Sidhu G, Maurus R, Rydberg EH, Braun C, Wang Y, *et al*. Subsite mapping of the human pancreatic α-amylase active site through structural, kinetic, and mutagenesis techniques. Biochemistry. 2000; 39: 4778–4791.

[38] Aghraz A, Gonçalves S, Rodríguez-Solana R, Dra LA, Di Stefano V, Dugo G, *et al*. Antioxidant activity and enzymes inhibitory properties of several extracts from two Moroccan Asteraceae species. South African Journal of Botany. 2018; 118: 58–64.

[39] Mourad B, Sihem B. Antioxidant Activity and Phenolic Content of *Artemisia campestris* from Two Regions of Algeria. World Journal of Environmental Biosciences. 2014; 7: 61–66.

[40] Megdiche-Ksouri W, Trabelsi N, Mkadmini K, Bourgou S, Noumi A, Snoussi M, *et al*. *Artemisia campestris* phenolic compounds have antioxidant and antimicrobial activity. Industrial Crops and Products. 2015; 63: 104–113.

[41] Dib I, Tits M, Angenot L, Wauters JN, Assaidi A, Mekhfi H, *et al*. Antihypertensive and vasorelaxant effects of aqueous extract of *Artemisia campestris* L. from Eastern Morocco. Journal of Ethnopharmacology. 2017; 206: 224–235.

[42] Alyahya HK, Subash-Babu P, Salamatullah AM, Hayat K, Albader N, Alkaltham MS, *et al*. Quantification of Chlorogenic Acid and Vanillin from Coffee Peel Extract and its Effect on α-Amylase Activity, Immunoregulation, Mitochondrial Oxidative Stress, and Tumor Suppressor Gene Expression Levels in H_2O_2-Induced Human Mesenchymal Stem Cells. Frontiers in Pharmacology. 2021; 12: 760242.

[43] Lo Piparo E, Scheib H, Frei N, Williamson G, Grigorov M, Chou CJ. Flavonoids for Controlling Starch Digestion: Structural Requirements for Inhibiting Human α-Amylase. Journal of Medicinal Chemistry. 2008; 51: 3555–3561.

[44] Miao M, Jiang H, Jiang B, Li Y, Cui SW, Zhang T. Structure elucidation of catechins for modulation of starch digestion. LWT - Food Science and Technology. 2014; 57: 188–193.

[45] Tadera K, Minami Y, Takamatsu K, Matsuoka T. Inhibition of alpha-glucosidase and alpha-amylase by flavonoids. Journal of Nutritional Science and Vitaminology. 2006; 52: 149–153.

[46] Lim J, Ferruzzi MG, Hamaker BR. Structural requirements of flavonoids for the selective inhibition of α-amylase versus α-glucosidase. Food Chemistry. 2022; 370: 130981.

[47] Schofield P, Mbugua DM, Pell AN. Analysis of condsensed tannins: a review. Animal Feed Science and Technology. 2001; 91: 21–41.

[48] Thinkratok A, Supkamonseni N, Srisawat R. Inhibitory Potential of the Rambutan Rind Extract and Tannin against Alpha-Amylase and Alpha-Glucosidase Activities *in vitro*. International Conference on Food, Biological and Medical Sciences. 2014; 2014: 28–29.

[49] Kandra L, Gyémánt G, ZajáczA, Batta G. Inhibitory effects of tannin on human salivary α-amylase. Biochemical and Biophysical Research Communications. 2004; 319: 1265–1271.

[50] C. Sridhar SN, Kumari S, T. Paul A. Diabetic Complications: a Natural Product Perspective. Pharmaceutical Crops. 2014; 5: 39–60.

[51] Giacco F, Brownlee M. Oxidative Stress and Diabetic Complications. Circulation Research. 2010; 107: 1058–1070.

[52] Tangvarasittichai S. Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. World Journal of Diabetes. 2015; 6: 456.

[53] Ahmed-Laloui H, Zaak H, Rahmani A, Kashi I, Chemat S, Miara MD, *et al*. Assessment of artemisinin and antioxidant activities of three wild Artemisia species of Algeria. Natural Product Research. 2022. (in press)