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*Original Article*

# *Ammodaucus leucotrichus* Coss. & Durieu: Antihyperglycemic activity via the inhibition of α-amylase, α-glucosidase, and intestinal glucose absorption activities and its chemical composition

[*Ammodaucus leucotrichus* Coss. & Durieu: Actividad antihiperglucémica por inhibición de α-amilasa, α-glucosidasa, y las actividades de absorción de glucosa intestinal y su composición química]

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**Abstract**

*Context*: *Ammodaucus leucotrichus* commonly known as a 'Kamune es sufi or akâman' in Morocco, is used to treat many diseases including diabetes.

*Aims*: To investigate the antihyperglycemic activity of an aqueous extract of fruits *A. leucotrichus* (AEAL) and its chemical composition.

*Methods*: The antihyperglycemic effect of the AEAL was tested against intestinal α-glucosidase and pancreatic α-amylase activities, *in vitro*, at the concentrations (41-328 µg/mL) and (0.5-3 mg/mL) respectively. In addition, the inhibitory effect of the AEAL (150 mg/kg) against these enzymes was confirmed, *in vivo* in normal and alloxan diabetic rats using sucrose, starch, and glucose as a substrate. The antihyperglycemic effect of the AEAL was also tested against intestinal D-glucose absorption activity at the dose of 150 mg/kg using the jejunum segment perfusion technique, *in situ*. Chemical composition was evaluated using HPLC.

*Results*: The results of this study showed that the AEAL was significantly ($p<0.001$) inhibited the intestinal α-glucosidase, *in vitro* ($IC_{50}$ = 0.254 mg/mL). The same effect of this extract was confirmed against the pancreatic α-amylase activity, with $IC_{50}$ = 1.81 mg/mL. *In vivo*, the oral intake of the AEAL at a dose of 150 mg/kg was significantly attenuated the hyperglycemia induced by the sucrose, starch, and glucose in the normal and alloxan diabetic rats. AEAL, also, significantly ($p<0.01$) decreased intestinal glucose absorption, *in situ.* HPLC results revealed the presence of four molecules: vanillin, quercetin, kaempferol, and thymol.

*Conclusions*: *A. leucotrichus* showed a significant antihyperglycemic activity. This effect can be explained by the inhibition of α-amylase, α-glucosidase activities, and the intestinal absorption of D-glucose.

*Keywords*: alloxan; α-glucosidase; α-amylase; *Ammodaucus leucotrichus*; anti-hyperglycemic: intestinal glucose absorption.

**Resumen**

*Contexto*: *Ammodaucus leucotrichus* comúnmente conocido como un 'Kamune es sufi o akâma' en Marruecos, se utiliza para tratar muchas enfermedades, incluida la diabetes.

*Objetivos*: Investigar la actividad antihiperglicémica de un extracto acuoso de frutos *A. leucotrichus* (AEAL) y su composición química.

*Métodos:* Se probó el efecto antihiperglucémico del AEAL contra las actividades de α-glucosidasa intestinal y α-amilasa pancreática, *in vitro*, en las concentraciones (41-328 µg/mL) y (0,5-3 mg/mL) respectivamente. Además, el efecto inhibidor del AEAL (150 mg/kg) contra estas enzimas, *in vivo* en ratas diabéticas normales y aloxan utilizando como sustrato sacarosa, almidón y glucosa. El efecto antihiperglucémico del AEAL también se probó contra la actividad de absorción de D-glucosa intestinal a la dosis de 150 mg/kg utilizando la técnica de perfusión del segmento de yeyuno in situ. La composición química se evaluó mediante HPLC.

*Resultados*: Los resultados de este estudio mostraron que el AEAL fue inhibido significativamente ($p<0,001$) la α-glucosidasa intestinal, *in vitro* ($IC_{50}$ = 0,254 mg/mL). El mismo efecto de estos extractos se confirmó contra la actividad pancreática de la α-amilasa, con $IC_{50}$ = 1,81 mg/mL. *In vivo*, la ingesta oral del AEAL a dosis de 150 mg/kg atenuó significativamente la hiperglucemia inducida por la sacarosa, el almidón y la glucosa en ratas diabéticas normales y aloxan. AEAL también disminuyó ($p<0,01$) la absorción de glucosa intestinal, *in situ*. Los resultados de HPLC revelaron la presencia de cuatro moléculas: vainillina, quercetina, kaempferol y timol.

*Conclusiones*: *A. leucotrichus* mostró una actividad antihiperglucémica significativa. Este efecto puede explicarse por la inhibición de las actividades de α-amilasa, α-glucosidasa, y la absorción intestinal de D-glucosa.

*Palabras Clave*: aloxano; α-glucosidasa; α-amilasa; *Ammodaucus leucotrichus;* antihiperglucémico; absorción glucosa intestinal.

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## INTRODUCTION

The global diabetes prevalence in 2019 is estimated to be 9.3% (463 million people), increasing to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045 (Saeedi et al., 2019). Diabetes mellitus is a metabolic disease due to endocrine dysfunction. It is characterized by a chronic rise in blood glucose, caused by absent insulin secretion and/or insulin resistance (Ozougwu et al., 2013). Several studies have shown that in diabetics, postprandial hyperglycemia increases the risk of microvascular and macrovascular complications (D'Amico et al., 2001). Persistent hyperglycemia affects the production and sensitivity of insulin, induces oxidative stress, and causes non-enzymatic glycosylation of different proteins (Tekin et al., 2011). Reduction of postprandial glycemia is important in the treatment of type II diabetes to prevent vascular complications (Shim et al., 2003).

One of the approaches to controlling postprandial hyperglycemia is inhibitions of α-amylase and intestinal α-glucosidase and reduced intestinal absorption of glucose. α-Amylase liberates maltose and glucose from starch by hydrolysis of α-(1,4)-glucosidic linkages, while α-glucosidase liberates glucose from maltose and/or sucrose (Nair et al., 2013). One therapeutic option to treat diabetes is to retard the absorption of glucose via inhibition of these enzymes (Wang et al., 2010; Ghosh et al., 2014).

Treatment options for diabetes possess several side effects and are costly. Therefore, the search for natural antihyperglycemic agents from medicinal plants has gained attention due to their safety and availability.

*Ammodaucus leucotrichus* Coss. & Durieu is an aromatic plant belonging to the *Apiaceae* family, its local name is 'Kamune es sufi or akâman', distributed in the maritime sands of the Saharan and sub-Saharan countries of North Africa; Morocco, Algeria, and Tunisia expanding to Egypt and tropical Africa (Tejera, 1983). In traditional therapy, *A. leucotrichus* fruits are commonly used as sugar regulators for diabetics, stomach, and colon diseases. The seeds are used to treat stomach diseases, wounds infections, cutaneous allergies, genital disorders, abdominal pains, scorpion stings, snakebites, and liver diseases. The leaves are used infused or used as a powder to avoid indigestion, to recover the appetite, and for chest complaints (Mohammedi et al., 2018). The leaves and seeds are used in the form of decoction or infusion for several therapeutic cases, such as blood pressure, chest pain, liver and digestive system ailments, gastroenteritis, as also for diabetes (Ziani et al., 2019). Furthermore, a pharmacological study realized showed that *A. leucotrichus* fruits had a significant effect on diabetes (El-Ouady and Eddouks, 2019). Currently, no study has been carried out on its action mechanisms. Hence, in the present work, we evaluate the effect of *A. leucotrichus* fruits aqueous extract (AEAL) on pancreatic α-amylase, intestinal α-glucosidase (*in vitro* and *in vivo*) and also monitored its effect on postprandial blood glucose level in normal and diabetic rats. In addition, its chemical composition using high-performance liquid chromatography (HPLC) was evaluated.

## MATERIAL AND METHODS

### Chemicals

The sucrose and the starch powders, α-glucosidase and α-amylase enzymes, dinitrosalicylic acid, acarbose, phlorizin dehydrate, D(+) glucose anhydrous, potassium chloride [KCl], magnesium chloride-6-hydrate [$MgCl_2.6H_2O$], and sodium chloride [NaCl] were purchased from Sigma-Aldrich. Alloxan monohydrate (Allx monohydrate 98%) was purchased from ACROS Organics. Pentobarbital was obtained from CEVA Santé Animale, France. Calcium chloride dihydrate [$CaCl_2.2H_2O$] (Scharlau Chemie S.A., Spain), sodium hydrogen carbonate [$NaHCO_3$], and citric acid were purchased from Farco Chemical Supplies, Puerto Rico). Sodium phosphate monobasic 2-hydrate [$NaH_2PO_4.2H_2O$] (Panreac, Spain), and glibenclamide were obtained from a local pharmacy (Oujda, Morocco) as benclamide 5 mg. Ether obtained from Somaprol, Casablanca, Morocco.

### Plant identification

Fruits of *Ammodaucus leucotrichus* Coss. & Durieu (*Apiaceae*) were bought from the herb market in Oujda (Oriental Morocco). The herb sample was deposited in the Herbarium, Department of Biology, Mohamed First University, Faculty of Sciences, Oujda, Morocco and identified by a botanist under the reference number (HUMPOM432).

### Extraction method

The aqueous extract of fruits was prepared by infusion of 20 g grounded of fruits and dried fruits in 400 mL of boiling distilled water for 30 min. The extract was then filtrated using filter paper and the extract was evaporated to dryness with a rotary evaporator and conserved at -20°C until use.

**Antihyperglycemic effects of *A. leucotrichus* fruits extract**

*Animals*

Male and female albino Wistar rats (170-250 g) and Swiss albino mice (20-30 g) were obtained from animal house maintained at our department. They were kept under conditions of constant temperature (22 ± 2°C) with a standard 12-h light:12-h dark cycle and free access to food and water. All animals were cared for in compliance with the Guide for the Care and Use of Laboratory Animals, US National Institutes of Health (2012). Experiments were performed according to the protocol approved by the Institutional Animal Care and Use Committee at the Faculty of Sciences Oujda under the certification number 15/19-LBBEH-06.

*Diabetes induction*

After one week of acclimatization, the rats were subjected to a 16 h fast. Experimental diabetes was induced according to the protocol described by Prince et al. (1998) with some modification. Diabetes was induced by a single intraperitoneal injection (i.p.) (150 mg/kg of body weight) of alloxan monohydrate dissolved in fresh and cold phosphate citrate buffer pH = 4.5. Animals with blood glucose levels of more than 1.26 g/L were included in the experiment.

*Oral Glucose Tolerance Test (OGTT)*

The Oral Glucose Tolerance Test (OGTT) of AEAL was evaluated as described by Chakravarty and Kalita (2012) with some modifications. The test was performed on normal and alloxan diabetic rats fasted for 16 h with free access to water. Rats were allocated into three groups with 6 animals in each group (3 males and 3 females). Normal diabetic rats received AEAL (150 mg/kg) orally, normal and diabetic rats received glibenclamide (2 mg/kg), and normal and diabetic controls received distilled water (10 mL/kg). All the animals were given the appropriate treatments 30 min before they received an oral glucose overload. Blood was taken before treatment (0 min), before glucose overload (30 min), and after glucose overload (90, 150 and 210 min), and blood glucose levels were estimated (Pandit et al., 2010).

*In vitro a-amylase inhibitory activity*

The α-amylase inhibitory activity of AEAL was determined based on the spectrophotometric assay using the 3,5-dinitrosalicylic acid (DNSA) method (Miller, 1959). The AEAL was dissolved in phosphate buffer (pH 6.9) to give concentrations from 0.5, 1, 1.5, 2, and 3 mg/mL. The enzyme α-amylase solution (13 UI/mL) was prepared by mixing 13 mg of α-amylase in 10 mL of 40 mM phosphate buffer. A volume of 200 µL of α-amylase was added to each test tube and incubated at 37°C for 10 min. Then 200 µL of 1% starch solution was added to each test tube and the mixture was re-incubated for 15 min at 37°C. The reaction was stopped by the addition of 600 µL DNSA reagent (12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM of 3,5-dinitrosalicylic acid solution). Then was boiled for 8 min in a boiling water bath. The mixture was cooled to ambient temperature and was diluted with 1 mL of distilled water, and the absorbance was measured at 540 nm. The blank with 100% enzyme activity was prepared by replacing the extract with 200 µL of the phosphate buffer. A blank reaction was similarly prepared using the plant extract at each concentration in the absence of the enzyme solution. A positive control sample was prepared using acarbose (0.5, 1, 1.5, 2, and 3 mg/mL) and the reaction was performed similarly to the reaction with plant extract as mentioned above. The α-amylase inhibitory activity was expressed as percent inhibition and was calculated using the equation [1].

$$\% \text{ Inhibition} = [(OD_{control} - OD_{treatment}/OD_{control})] \times 100 \quad [1]$$

*In vitro a-glucosidase inhibitory activity*

Alpha-glucosidase (0.1 U/mL) was dissolved in 100 mM phosphate buffer (pH 7.5), and 0.1 mL of sucrose (50 mM) was used as a substrate. A volume of 20 µL (41, 82, 246, and 328 µg/mL) of *A. leucotrichus* extract and the same volume of distilled water and acarbose as a control and negative and positive controls were used, respectively. The mixture was incubated at 37°C for 20 min, and then the reaction was interrupted by heating at a temperature of 100°C for 5 min. Absorbance was measured at 500 nm by spectrophotometry (UV-1800 UV/VIS Spectrophotometer) (Kim et al., 2000). The concentration of glucose liberated from the reaction was determined by the glucose oxidase method using a commercial kit. The percentage of inhibition was calculated according to the formula [1].

*In vivo a-amylase and a-glucosidase inhibitory activity*

To affirm the inhibitory effect of AEAL on α-amylase and α-glucosidase *in vivo*, an experimental design was described by Ortiz-Andrade et al. (2005) and Subramanian et al. (2008) with some modifications (Daoudi et al., 2020). The rats were subjected to fasting for 16 h before the experiment but with free access to water. AEAL was dissolved in distilled water. The animals were divided into three groups with 6 rats in each (n = 6; ♂/♀ = 1). Groups 1 received distilled water (10 mL/kg). Groups 2 were fed by AEAL

at 150 mg/kg (the minimum dose that gives significant results, determined after preliminary testing). Group 3 received acarbose at 10 mg/kg. Thirty min after oral administration of distilled water, acarbose, or AEAL, the rats were orally administered with 3 g/kg starch or 2 g/kg sucrose. Blood was collected in 0, 30, 60, and 120 min after administration of sucrose and starch to measure blood glucose concentration. The glucose concentration was determined by the method of glucose oxidase peroxidase (Trinder, 1969).

*Intestinal glucose absorption, in situ*

Intestinal glucose absorption was evaluated according to the protocol described by Ponz et al. (1979) with some modification, using the jejunum segments perfusion technique. The test was assessed in normal Wistar rats fasted for 36 h, with free access to water. Rats (170-250 g) were divided into 3 groups with 6 rats in each (n = 6; ♂/♀ = 1): group 1 served as control and received the perfusion solution; group 2 served as positive control and received the solution with added phlorizin (0.1 mM) and group 3 received the solution with added 150 mg/kg of AEAL (the same dose used in the oral glucose tolerance test). The composition of the perfusion solution was (g/L): 7.37 NaCl, 0.2 KCl, 0.065 $NaH_2PO_4.2H_2O$, 0.213 $MgCl_2.6H_2O$, 0.6 $NaHCO_3$, and 1.02 $CaCl_2.2H_2O$. D-glucose (1 g/L) was added to the solution just before the start of the experiment and the pH was maintained at 7.5. Rats were anesthetized by intramuscular injection of sodium pentobarbital (50 mg/kg) and fixed on a homoeothermic bath to keep the body temperature at 37°C. A jejunum segment (10 cm) was catheterized with polyethylene tubes at both proximal and distal ends. After catheterization, the incision was sutured, and the surgical area was covered with cotton imbibed with saline (0.9% NaCl) to prevent dehydration. After 1 h, the perfusate was collected from a polyethylene tube at proximal ends and glucose concentration was measured using the glucose oxidase peroxidase method by a commercial kit (Barcelona, Spain). The length of the perfused jejunal segment was measured and the intestinal glucose absorption was estimated in mg/10 cm/1 h.

**High-performance liquid chromatography analysis**

High-performance liquid chromatography (HPLC) analysis was carried out on an Agilent 1100 Series equipped with Diode Array Detector (HPLC-DAD), manual injection, quaternary pump, thermostatted column compartment, and a C18 Hypersil BDS column (250 × 4.6 mm; 5 µm). The temperature was kept stable at 30°C during the analysis. Using a solution (A) of ultra-purified water + acetic acid (0.2%) (v/v) and an acetonitrile solution (B) at a flow rate of 1 mL/min, HPLC analysis started with 95% of A followed by a linear gradient to 100% of B for 40 min. Chromatograms were obtained after eluting 15 µL of samples and standards injected at 300 nm because most phenolic compounds show reasonably high absorbance at this wavelength value (Seo and Morr, 1984).

**Acute toxicity**

Acute oral toxicity study was evaluated following the recommendations by OECD Guidelines (425) (In , 2001). Oral acute toxicity was realized on albino mice weighing 20-30 g, using a single dose, which was administered orally. For each route, four groups of six mice (3 males and 3 females) received increasing doses of AEAL (1, 2, 5 g/kg) or distilled water (10 mL/kg). After treatment, the animals were observed for 14 days to assess behavioral changes and signs of toxicity and/or death and the latency of death.

**Statistical analysis**

The results obtained have been analyzed by Graph Pad Prism 8.4.3 and been expressed as mean ± standard error of the mean (SEM). The results have been analyzed by Two-way ANOVA, followed by Bonferroni's multiple comparisons test. The difference was considered statistically significant when $p<0.05$.

## RESULTS

**Effect of AEAL on Oral Glucose Tolerance Test (OGTT), in normal and diabetic rats**

Oral administration of AEAL at 150 mg/ kg to normal rats significantly inhibited ($p<0.001$) the hyperglycemia that follows glucose loading, 0.07 ± 0.11 g/L at 90 min for treated rats and 1.81 ± 0.15 g/L for a normal control group (Fig. 1A). In the Allx-diabetic rats (Fig. 1B), oral administration of AEAL improved the glucose tolerance in the Allx-diabetic treated group (2.04 ± 0.18 g/L) at 90 min, compared with the diabetic control group (3.88 ± 0.12 g/L). Glibenclamide significantly ($p<0.001$) decreased the blood glucose level at 90 min in normal and diabetic rats.

***In vitro* α-amylase inhibitory activity**

The Fig. 2 showed that AEAL was significantly inhibited the α-amylase activity, *in vitro*, as compared to the control with $IC_{50}$ values of 1.81 ± 0.03 mg/mL. The AEAL concentration of 3 mg/mL showed a more active effect than the other concentrations with inhibitory activities of 68.13% compared to acarbose (78.73%).

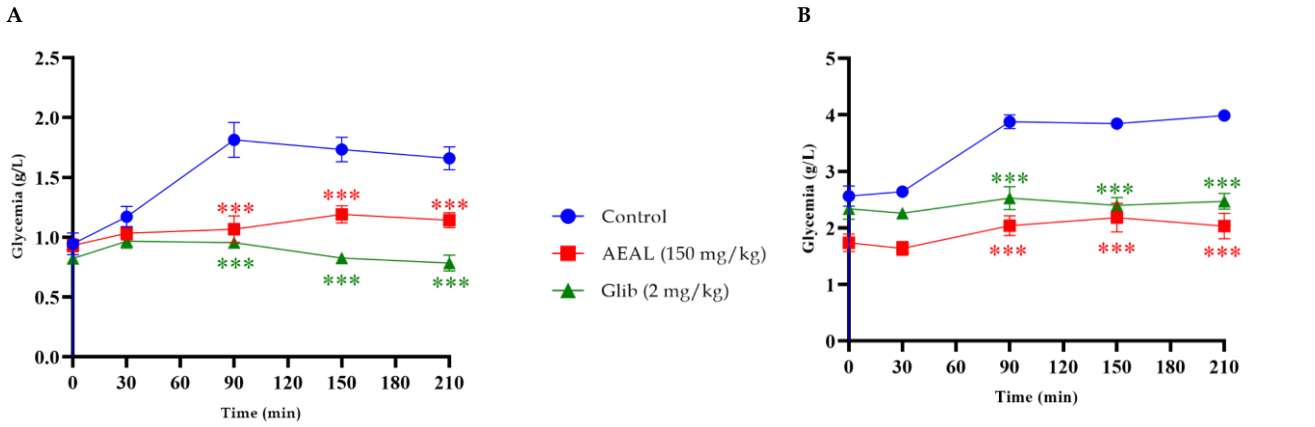


**Figure 1.** Effect of the aqueous fruits extract of *A. leucotrichus* (AEAL) on glycemia after glucose loading in normal **(A)** and diabetic rat **(B)**.
Data represent mean ± standard error of the mean (n = 6). ***p<0.001 as compared to the control. Glib: glibenclamide.

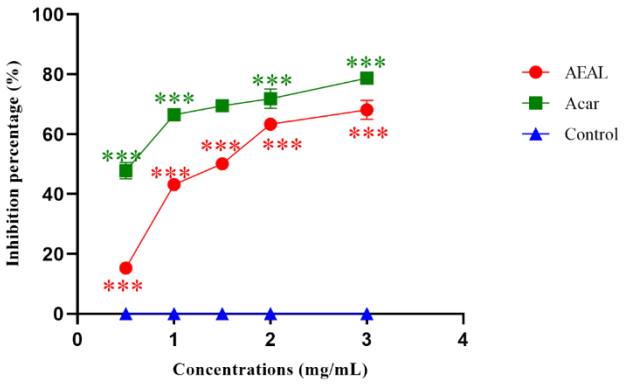


**Figure 2.** Inhibitory activity of the aqueous fruits extract of *A. leucotrichus* (AEAL) and acarbose (positive control) against α-amylase at different doses (0.5, 1, 1.5, 2 and 3 mg/mL).
Data represent mean ± standard error of the mean (n = 6). ***p<0.001 as compared to the control. AEAL: aqueous fruits extract of *A. leucotrichus* and Acar: acarbose.

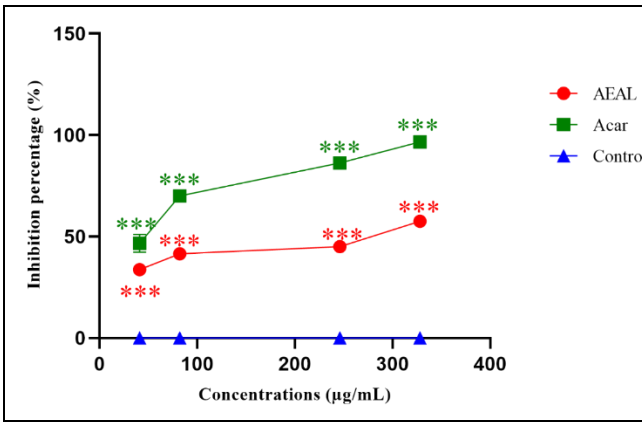


**Figure 3.** Inhibitory activity of the aqueous fruits extract of *A. leucotrichus* (AEAL) against α-glucosidase at four different concentrations (41, 82, 256 and 328 µg/mL).
Data represent mean ± standard error of the mean (n = 6). ***p<0.001 as compared to the control. Acar: acarbose.

### *In vitro* α-glucosidase inhibitory activity

The results showed that AEAL was significantly inhibited (p<0.001) the activity of the α-glucosidase *in vitro*, as compared with the control (Fig. 3) with $IC_{50}$ values of 0.254 ± 0.004 mg/mL. The most active concentration of the AEAL was 328 mg/mL with an inhibitory percentage of 57.56 ± 1.37 %.

### *In vivo* α-amylase inhibitory activity

The results revealed that in the control group postprandial hyperglycemia increased to 1.4 g/L, 30 min after starch administration (Fig. 4A). However, in the presence of 150 mg/kg of AEAL, this postprandial hyperglycemia caused by starch decreased significantly (p<0.01) at 30 min. Oral administration of acarbose to normal rats 30 min before oral administration starch (3 g/kg body weight), as expected, significant-

ly deleted the rise in postprandial hyperglycemia at 30 and 60 min in these rats.

The Fig. 4B represents the effect of AEAL on glycemia after oral starch overload in Allx-diabetic rats. The results showed that in the diabetic control group blood glucose increased from 1.3 to 1.9 g/L at 30 min after oral starch overload. However, in the presence of 150 mg/kg of AEAL, this postprandial hyperglycemia decreased significantly ($p<0.01$ and $p<0.001$) at 30 min compared to the control. Oral administration of acarbose to diabetic rats 30 min before oral administration starch, significantly ($p<0.001$) decreased the postprandial hyperglycemia at 30 and 60 min.

### *In vivo* α-glucosidase inhibitory activity

The results showed that in the control group postprandial hyperglycemia increased to 1.40 g/L, 30 min after sucrose administration, and then decreased to 1.34 g/L, 120 min after. However, in the presence of 150 mg/kg of AEAL, this postprandial hyperglycemia caused by sucrose decreased significantly ($p<0.01$, $p<0.001$, and $p<0.001$, respectively) at 30, 60, and 120 min. Acarbose at 10 mg/kg significantly ($p<0.001$) decreased blood glucose 30 min after sucrose administration (Fig. 5A).

Fig. 5B showed that in the diabetic control group blood glucose increased from 2.17 to 3.7 g/L at 30 min after oral sucrose overload. However, in the presence of 150 mg/kg of AEAL, postprandial blood sugar increased from 1.8 to 2.1 g/L at 30 min after sucrose overload with a significant decrease ($p<0.001$) compared to the diabetic controls.

### Intestinal glucose absorption, *in situ*

The results (Fig. 6) revealed that the amount of glucose absorbed in the jejunum in the absence of AEAL (Control group) is 10.72 mg/10 cm/h. However, in the presence of the AEAL at a dose of 150 mg/kg, the amounts of the intestinal glucose uptake decrease significantly as compared with the control, with an amount of 6.12 ($p<0.01$). Phlorizin (0.1 mM) showed an important reduction in intestinal absorption with a quantity of 3.24 mg/10 cm/h ($p<0.001$).

### HPLC analysis

The identification of compounds in the aqueous extract from fruits of *A. leucotrichus* was performed by HPLC using UV/visible (DAD). Peak detection is initially achieved by comparing the unknown component's retention time to that of the standard. The obtained chromatograms were shown in Fig. 7. As a result, several peaks appeared at 300 nm, four peaks were identified: (1) vanillin, (2) quercetin, (3) kaempferol, and (4) thymol.

### Acute toxicity

Oral administration of AEAL exhibited no mortality, no signs of toxicity, and no changes in general behavior or motor disorder in mice at all tested doses.

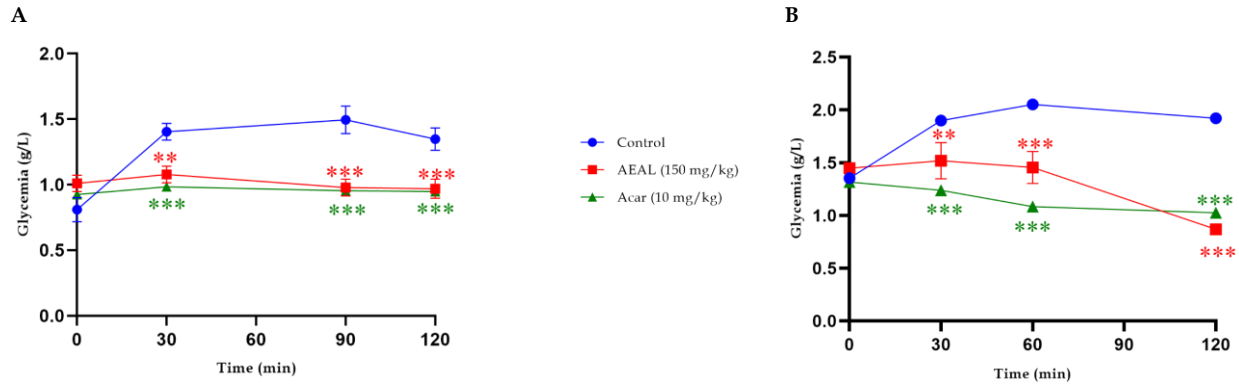


**Figure 4.** Effect of the aqueous fruits extract of *A. leucotrichus* (AEAL) on glycemia after starch loading in normal **(A)** and Allx-diabetic rats **(B)**.

Data represent mean ± standard error of the mean (n = 6). **p<0.01; ***p<0.001 *versus* control. Acar: acarbose.

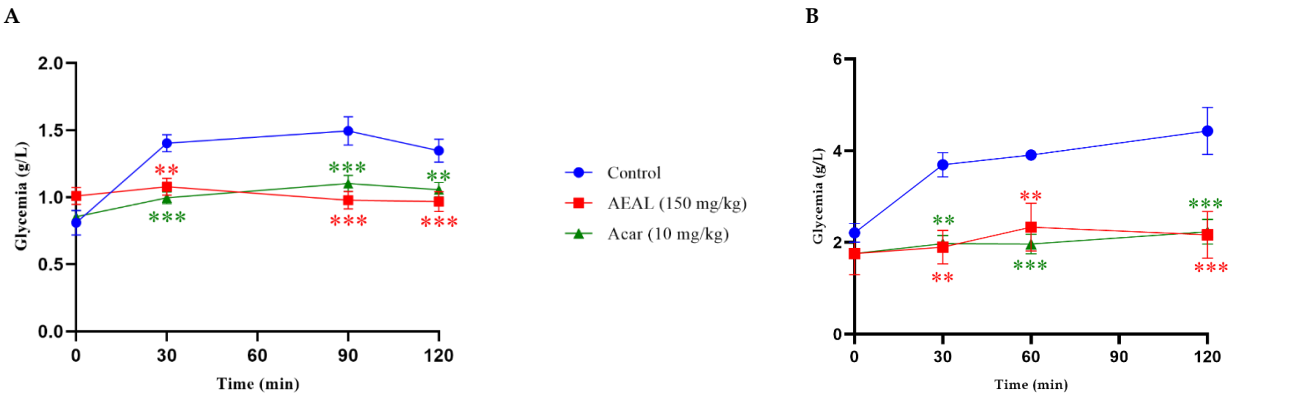


**Figure 5.** Effect of the aqueous fruits extract of *A. leucotrichus* (AEAL) on glycaemia after sucrose surcharge in normal **(A)** and diabetic rats **(B)**.

Data represent mean ± standard error of the mean (n = 6). **p<0.01; ***p<0.001 *versus* control. Acar: acarbose.

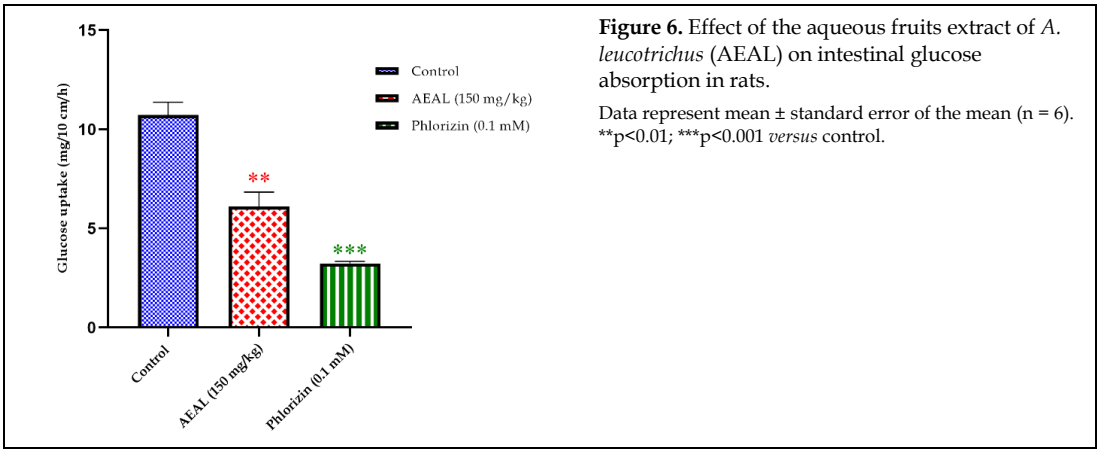


**Figure 6.** Effect of the aqueous fruits extract of *A. leucotrichus* (AEAL) on intestinal glucose absorption in rats.

Data represent mean ± standard error of the mean (n = 6). **p<0.01; ***p<0.001 *versus* control.

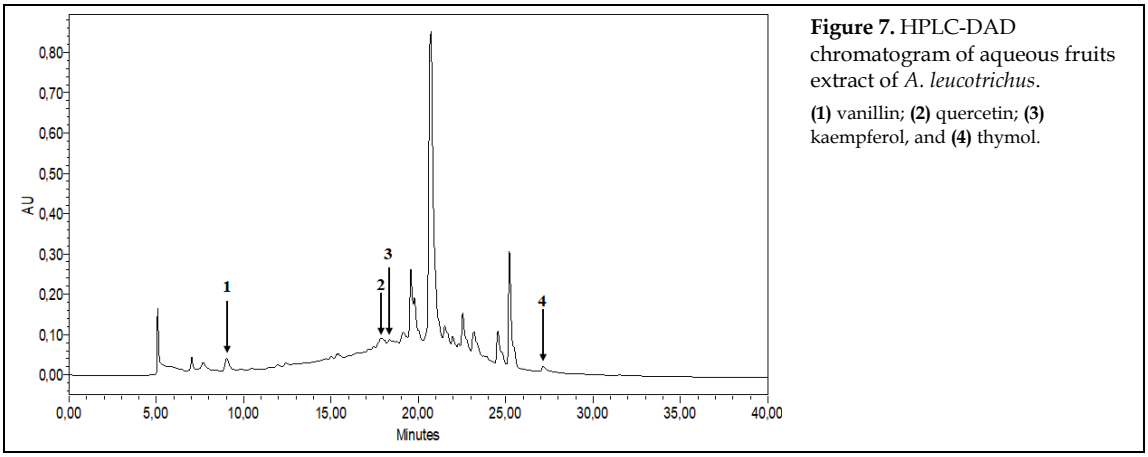


**Figure 7.** HPLC-DAD chromatogram of aqueous fruits extract of *A. leucotrichus*.

**(1)** vanillin; **(2)** quercetin; **(3)** kaempferol, and **(4)** thymol.

## DISCUSSION

This work investigates the antihyperglycemic effect of *A. leucotrichus* in normal and Allx-induced diabetic rats. Normal and Allx-diabetic rats were used in oral glucose tolerance tests, all of which were overloaded with glucose. This model was chosen to evaluate the action of an AEAL administration on postprandial blood sugar induced by carbohydrate overload. The results reveal that oral administration of AEAL can improve glucose tolerance by decreasing postprandial hyperglycemia in normal and diabetic rats (Fig. 1A-B). Various mechanisms are implicated in the regulation of postprandial glycemia. In this study, the attenuation of the hyperglycemic peak by AEAL could result from the inhibition of α-amylase, α-glucosidase, and intestinal glucose absorption activities, which are considered as the best alternative for postprandial hyperglycemia management and one of

the strategies adopted to control diabetes (Mccue et al., 2005; Telagari and Hullatti, 2015).

This study based on *in vitro, in situ,* and *in vivo* examinations, *in vitro* assay, provide to directly evaluate the effect of AEAL on the activity of α-glucosidase and α-amylase enzymes. Although, *in vitro* assay is not enough and must be confirmed by other assays. For that, these activities were confirmed *in vivo* in normal and Allx-diabetic rats. The results of this study showed that the AEAL inhibits significantly the α-amylase and α-glucosidase activities *in vitro* (Figs. 2 and 3) and *in vivo* (Figs. 4 and 5) and they can lower the intestinal glucose absorption (Fig. 6). This affirms that the active compounds responsible for the antihyperglycemic activity of the *A. leucotrichus* fruits are extractable in water, which sustains the traditional uses of *A. leucotrichus* in the treatment of diabetes. The HPLC analysis revealed the presence of four polyphenolic compounds (Table 1): vanillin, quercetin, kaempferol, and thymol. Some of them are well known for their antihyperglycemic effect namely thymol (Agarwal et al. 2020; Oskouei et al. 2019), kaempferol (Ibitoye et al., 2018). Moreover, kaempferol has potent inhibitory activity against α-amylase and α-glucosidase (Sheng et al. 2018), and can decrease glucose intestinal absorption (Rodríguez et al. 2010). Another study realized by Louail et al. (2020) revealed the presence of ferulic acid in *A. leucotrichus*. Furthermore, ferulic acid also plays a role as α-amylase and α-glucosidase inhibitors (Zheng et al., 2020).

In addition, *A. leucotrichus* revealed a high concentration in flavonoids, due to the presence of luteolin derivatives, such as luteolin-7-O-glucoside and luteolin-O-(malonyl-hexoside) isomer 2 (Ziani et al., 2019). Luteolin inhibitory effect on α-glucosidase and α-amylase has been demonstrated by Kim et al. (2000). Polyphenols are recognized for their wellness benefits, they exert their antidiabetic action via numerous pathways and multiple molecular targets by controlling the signaling, secretion, and action of insulin, regulating the digestion of carbohydrates, and the control of glucose level in the body. They also regulate the activity of β-cells by promoting their proliferation and insulin secretion (Graf et al., 2005; Vinayagam and Xu, 2015).

Evaluation of the acute toxicity of the aqueous extract of *A. leucotrichus* confirms that its use is healthy and without adverse effects under the experimental conditions carried out.

## CONCLUSION

This study investigated the potential antihyperglycemic activity of the *A. leucotricus*, focusing on the inhibitory effects on α-glucosidase, α-amylase, and intestinal glucose uptake. This study is the first to report a potential, mechanism of action of *A. leucotricus* and suggests that the antihyperglycemic effect of this plant is due to the inhibition of digestive enzymes, α-glucosidase, α-amylase, and lowering the intestinal glucose absorption. In conclusion, the results from this study give scientific support to the use of *A. leucotricus* in traditional medicine for the treatment of diabetes mellitus. This study would be helpful to explain the pharmacological mechanism and contribute to the development of medicinal preparations, nutraceutical or functional foods for diabetes and related symptoms.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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| Contribution | Bouknana S | Daoudi NE | Bouhrim M | Ziyyat A | Legssyer A | Mekhfi H | Bnouham M |
|---|---|---|---|---|---|---|---|
| Concepts or ideas | | | | | | | x |
| Design | | | | | | | x |
| Definition of intellectual content | x | | | | | | |
| Literature search | x | x | x | | | | |
| Experimental studies | x | | | | | | |
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