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Original Research Article

Chemical profiling and pharmacological properties of *Arbutus unedo* L. collected from Morocco

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ABSTRACT

Arbutus unedo L. plant is traditionally used in Morocco to treat various ailments, in particular diabetes. The goal of this study was to evaluate the pharmacological potential of *Arbutus unedo* fruit and flower extracts in addressing postprandial hyperglycemia and inflammation by inhibiting of digestive enzymes and anti-inflammatory mechanisms. It also aims to identify the bioactive compounds linked to these effects. Therefore, the present study assessed the anti-hyperglycemic potential of *Arbutus unedo* fruit and flower extracts via inhibitory effects on α-amylase and α-glucosidase both *in vitro* and *in vivo* in normoglycemic rats, while anti-inflammatory activity was evaluated via erythrocyte membrane stabilization and protein denaturation inhibition. Bioactive compounds were identified using GC-MS and their molecular interactions were examined through docking studies. The outcomes of this research showed that the fruit extract, containing high levels of quinic acid (33.22%) and azulene (19.01%), displayed stronger anti-inflammatory activity than the flower extract. Notably, the ethanolic fruit extract exhibited potent *α*-amylase inhibition (p <0.001). The methanolic fruit extract showed high *in vivo α*-glucosidase inhibition compared to acarbose. Molecular docking revealed that a novel compound isolated from the fruit, (5E)-5-benzylidene-3-[(2-methoxyanilino)methyl]-1,3-thiazolidine-2,4-dione, had high binding affinity for both digestive enzymes.These findings position *Arbutus unedo* fruit as a promising natural therapeutic candidate for glycemic control and inflammation.

Keywords: *Arbutus unedo*, Digestive Enzymes, Inflammation, *In Vitro, In Vivo, In Silico*.

Introduction

Diabetes mellitus is chronic hyperglycemia due to insufficient insulin secretion and/or a defect in insulin function.[1] Worldwide, Type 2 diabetes mellitus (T2DM) is the most common form of diabetes, affecting men and women of all ages: children, youth and adults, representing 90% of diabetes cases .[2] Postprandial hyperglycemia is widely reported as a factor contributing to the progression of various diabetic complications.[3] According to the World Health Organization (WHO), the prevalence rate of diabetes among the adult population in Morocco is estimated at 12.4%.

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This disease causes more than 12,000 deaths per year and is responsible for an additional 32,000 deaths attributable to complications related to high blood glucose levels.[4] Scientific evidence has clearly demonstrated a strong link between inflammation and the development of diabetes-related complications, underlining the crucial role inflammation plays in the progression of these serious health problems .[5] Of particular interest, it has been reported that protein denaturation is strongly associated with the initiation and persistence of inflammation ,and is therefore seen as a marker of inflammatory diseases .[6,7] On the other hand, stabilization of the lysosomal membrane is essential for controlling the inflammatory response. It prevents activated neutrophils such as bactericidal enzymes, from releasing their lysosomal components into the extracellular environment, which helps prevent worsening of inflammation and tissue damage .[8] In fact, it has been observed that the lysosomal membrane is similar to that of erythrocytes.[9] As a result, it has been suggested that agents that stabilise red blood cell membranes may also stabilize the lysosomal membrane .[10] In addition, non-steroidal anti-inflammatory drugs act either by stabilizing the membranes of these lysosomes or inhibiting the release of their enzymes .[11]

One of the main therapeutic strategies currently used to manage postprandial hyperglycemia in type 2 diabetes is the inhibition of digestive enzymes such as alpha-amylase and alpha-glucosidase .[12] This approach aims to prolong the digestive process by slowing down the

breakdown, thereby reducing the absorption of carbohydrates .[13] However, these drugs are synthetic and are often associated with a number of adverse effects, and are subjects to the development of tolerance over time requiring regular dosage adjustments.[2] Consequently, most patients used folk medicine alone or as a complement to prescribed therapies. Thus, the use of medicinal plants for the treatment of diabetes is a widespread customary practice in various regions of Morocco, especially in rural areas and its use is increasing in urban ones. In this context, many medicinal plants, including *Arbutus unedo* L., are considered promising complementary or alternative therapies for the management of diabetes, due to their affordability and lack of adverse side effects. *A. unedo* L. (Straweberry tree) is an evergreen shrub known in Morocco as the ''sasnou''.It is widely widespread in the Mediterranean region, particularly in the forests of Morocco. Traditionally, their leaves and roots are used to treat a range of diseases, including diabetes, inflammation, hypertension, renal disorders, diarrhoea and cardiovascular dysfunctions.[14] Phytochemical studies on fruit extracts identified several biochemical components, including flavonoids, polyphenols and iridoids,while flower extracts revealed the presence of polyphenols .[15,16]
Research has mainly focused on the effect of the leaves and roots of this plant on hyperglycemia, while few reports have mentioned this effect in the fruit. We therefore set out to assess the anti-hyperglycemic potential of these fruits in an *in vivo* model; to our knowledge, no information is available on this type of study in this fruit and flower of *A.unedo*. Furthermore, no report has been published on the anti-inflammatory effect of these flowers. The objective of this study was therefore to unveil the effects of Moroccan *Arbutus unedo* fruit and flower extracts on postprandial hyperglycemia *in vitro* and *in vivo*, and in preventing the factors involved in the complications of diabetes with particular emphasis on their anti-inflammatory properties. In addition, the study aimed to identify the most important bioactive compounds associated with these activities by assessing the overall composition of various *A. unedo* L. extracts and using molecular-docking analysis.

Materials and Methods

Chemicals and reagents
HPLC grade solvents: 2-propanol was both from Scharlau, Spain. Methanol (CH_3OH) and water were acquired from Carl ROTH, Germany,and ethanol were obtained from VWR, France. Sucrose (purity ≥99.5%), soluble starch powder (food grade, analytical standard), and bovine serum albumin (BSA) have all been provided from Sigma-Aldrich, Germany.The standard α-glucosidase enzyme (from *Saccharomyces cerevisiae*, ≥10 units/mg protein) and acarbose (purity≥98%) were obtained from Sigma-Aldrich,China.The porcine pancreatic α-amylase (EC 3.2.1.1, ≥10 units/mg protein) was acquired from Sigma-Aldrich,USA.The glucose oxidase–peroxidase (GOD–POD) reagent kit was obtained from Biosystems, Spain, and used for enzymatic glucose determination.Sodium chloride (NaCl, ≥99.5%) was purshased from Fluka, Sigma-Aldrich, Switzerland. 3,5-Dinitrosalicylic acid (DNSA) (≥98%),used in quantifying reducing sugars, was purchased from Riedel-de Haën, Sigma-Aldrich,Germany.Aspirin (O-Acetylsalicylic acid, ≥99%) was bought from Fluka, Switzerland.Phosphate-buffered saline (PBS,10×concentrate) was imported from VWR, United Kingdom.N,O-Bis(trimethylsilyl)trifluoroacetamide) (BSTFA) + 10% trimethylchlorosilane (TMCS) were acquired from Thermo Scientific,USA. Chemicals were of analytical quality.

Plant material and extraction procedure
Fruits and flowers of *Arbutus unedo* (Ericaceae) were collected in the EL Harcha forest in the Rabat-Sale-Kenitra region of Morocco (latitude 33°28'31.296" N and longitude 6°8'50.568"), during the fruiting and flowering season in January 2022. The plant material was authenticated at the Scientific Institute of Mohammed V University in Rabat, Morocco, and the specimen was then archived in the institute's herbarium under reference number RAB114855. Samples were then subjected to a freeze-drying process, followed by grinding to a fine powder. Aqueous extracts were prepared by decoction under reflux for 30 min (25 g/250 mL (w/v)). The filtrate underwent vacuum evaporation via a rotary evaporator (Heidolph Scientific Products GmbH,Germany) at 60°C, and then was frozen at -20°C and freeze-dried (Labconco Corporation ,USA).[17] To prepare the hydroalcoholic extracts, 100 mL of various 50% (v/v) solvents (methanol, 2-propanol, ethanol) were added to 5 g of lyophilized powder, as described by Alexandre et al. in 2018.[18] The mixture was then shaken for 48 h. Extracts of plant materials were filtered, using fritted glass disk funnels to remove all residual particles, then reduced under vacuum using a rotary evaporator at 65°C.They were then collected and dried using a Duran™ desiccator (DWK Life Sciences™, Germany) and finally stored at -20 °C.

GC-MS Profiling analysis
Methanolic and ethanolic extracts from the fruits and flowers of *A. unedo* were analyzed by gas chromatography coupled with a mass spectrometry (GC/MS) (Brand Agilent Technologies Model 5972 ,USA). This instrument comprises a Hewlett-Packard 5890 II gas chromatograph fitted with an HP 5972 Mass selective detector and a VB-5 capillary column (5% phenyl; 95% methylpolisyloxane). Prior to GC/MS analysis, the extracts underwent a derivatization process to ensure the stability and volatility of the compounds for accurate detection. For derivatization, 1 mL of BSTFA + TMCS (90:10) was added to the extracts, followed by agitation for 30 minutes at 70°C. The injection volume was 1 µL in a capillary column 30 m long, 0.25 mm in diameter and with a film thickness of 0.25 µm. The injector temperature was maintained at a constant 250°C. MS detection was performed using an electron ionization system with an ionization energy of 70 eV. The carrier gas utilized was helium, with an inlet pressure of 10.48 psi and a flow rate of 1.0 mL/min. The oven temperature was increased from 60 °C to 280 °C in increments of 16 °C per minute, and then maintained at the final temperature for 10 minutes.The compounds present in *Arbutus unedo* were identified using the Wiley Registry 11th Edition /NIST 2017 Mass Spectral Library database.

Experimental animals
The study utilised 36 normoglycaemic rats with a weight range of 180-200 grams and rabbits with a weight range of 1.5-2 kilograms. The rats were obtained from the animal house of the Biology Department at the Faculty of Science in Oujda, while the rabbits came from the animal house at the Hassan II Agronomic and Veterinary Institute in Rabat. The animals were maintained under standard laboratory conditions at an ambient temperature of 24 ± 2°C, in a well-ventilated animal house with a 12-hour light/dark cycle, with food and water available. They were treated in accordance with the standards established by the National Institutes of Health guidelines for the treatment and use of laboratory animals .[19] The studies were conducted in accordance with a protocol approved by the Institutional Care and Use Committee of the Faculty of Sciences at Mohammed First University in Oujda, with the certification reference 15/19-LBBEH-06.

Antihyperglycemic potential
In vitro inhibitory effect on pancreatic α-amylase activity
The inhibitory activity of the extracts on α-amylase activity was determined as described in the method of Abdnim et al. in 2023 with minor modifications.[3] Specifically, 200 µL of phosphated buffer (0.2 M ; pH= 6.9) , 200 µL of α-amylase enzymatic solution (13 IU) and 100 µL of fruit and flower extracts (0.062, 0.12, 0.25, 0.5 and 1 mg/ml) or acarbose (0.45 to 4.5 mg/mL) were preincubated at 37°C for 10 minutes using a Shel Lab W20M water bath (Shel Lab, USA). Subsequently, 200 µL of a starch blend (1% dissolved in phosphate buffer) was added. Following a 20-minute incubation period at 37°C, 600 µL of 3,5-dinitrosalicylic acid coloured reagent was added to the reagent mixture. The tubes were then placed in a hot water bath at 100°C for 8 minutes, and then chilled in an ice-water bath for 5 minutes. The reagent blend was then diluted with 1 mL of distilled water, and the optical density measured at 540 nm. All samples were taken three times (n =3). The inhibiting activity of α-amylase was derived from the equation 1 given below:

$$\%\ \text{of Inhibition} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

Where:

$A_{control}$= Absorbance of the reaction without inhibitor (100% enzyme activity),
A_{sample}= Absorbance in the presence of either plant extract or acarbose.

In vitro inhibitory effect on intestinal α-glucosidase activity
The impact of the extracts on the enzyme activity of intestinal α-glucosidase was analyzed *in vitro*, according a previously described method, with slight modifications.[12] The method involves quantifying the D-glucose released by the breakdown of sucrose. To this end, 20 µL of different concentrations of acarbose solutions ranging from 0.45 to 4.5 mg/mL or fruit and flower extracts (0.062 to 1mg/mL) were added to a mixture consisting of 100 µL of 50 mM sucrose, 100 µL of an α-glucosidase enzyme solution (10 IU), and 1 mL of 50 mM phosphate buffer (pH 7.5).Subsequently, the mixtures were incubated in a water bath and maintained at 37°C for 25 min. After the incubation period, the different solutions were immediately heated to 100°C in a water bath for five minutes, in order to stop the enzymatic reaction. The control was prepared with the same reaction mixture, replacing the 20 µL of extracts with phosphate buffer. The amount of released D-glucose was determined by using a Glucose Colorimetric Assay (GOD/POD) kit, which relies on the ability of glucose oxidase to catalyze the oxidation of glucose to gluconic acid, producing hydrogen peroxide. Spectrophotometric measurements were performed at 500 nm using a spectrophotometer (model UV-160A, Shimadzu, Japan). The procedure was repeated three times for each sample (n=3), and the percentage of inhibition was then calculated as follows (equation 2):

$$\% \text{ of Inhibition} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

Where:
$A_{control}$= Absorbance of the reaction without inhibitor (100% enzyme activity),
A_{sample}= Absorbance in the presence of either plant extract or acarbose.

In vivo pancreatic α-amylase inhibitory assay
The *in vivo* pancreatic α-amylase inhibition test was conducted on four groups of rats (n=6; ♂/♀=1), who underwent a 16-hour fasting period. A dose of 10 mL/kg of distilled water was administered to group I, the control group .Group II was treated with a single 10 mg/kg dose of the standard drug, acarbose. Groups III and IV received a single dose of ethanolic extract and 2-propanol extract of the fruit at 150 mg/kg. All groups received a load of starch solution (2 g/kg) 30 minutes after the initial administration. All treatments were administered orally by intragastric gavage using a flexible gavage feeding sonde model FNC20-75 (Instech Laboratories, USA).[12] Blood glucose levels were then monitored at different time intervals (0, 30, 60, 90 and 120 minutes), using the glucose-peroxidase test with a GOD/POD kit.

In vivo intestinal α-glucosidase inhibitory assay
To determine the *in vivo* effect of the extracts on intestinal α-glucosidase, rats were fasted for 16 hours and randomly distributed into four groups of rats (n=6; ♂/♀=1). Control group I received an intragastric gavage of 10 mL/kg of distilled water. In contrast, the group II received a single dose of acarbose at 10 mg/kg. The remaining groups were given a single dose of either methanolic fruit extract or ethanolic flower extract at a dosage of 150 mg/kg. After 30 minutes following the administration of the solutions, sucrose was orally administered to the rats at a dose of 2 g/kg .[12] Glycemia levels were then measured at 0, 30, 60, 90 and 120 minutes, using the glucose-peroxidase method (GOD/POD kit).

Anti-Inflammatory potential assay, in vitro
Red blood cell membrane stabilization (RBCs)
The membrane-stabilizing test was performed according to the procedure described by Hasan et al. in 2019.[20] Specifically, blood was collected from a healthy male rabbit and transferred to an Eppendorf tube containing an anticoagulant (3.2 % in distilled water). The blood was then centrifuged (3,000 rpm) for 10 minutes and the packed cells meticulously washed with a 0.85% (w/v) NaCl solution. Conclusively, a suspension of packed cells at 10% (v/v) was prepared in 0.85% NaCl (w/v) solution and maintained at 4°C.The test was performed using the following reaction mixture: 1000 µL of phosphate buffer (0.15 M ; pH 7.4), 2000 µL of NaCl (0.36%, w/v), 500 µL of *Arbutus unedo* L. extract at varying concentrations (200 to 800 µg/mL), and 500 µL of the 10% erythrocyte suspension. Distilled water and aspirin were used as negative and positive controls, respectively. After incubation at 37°C for 30 minutes in an Isotemp Oven incubator (Fisher Scientific, USA) and centrifugation (Heraeus Biofuge Stratos model,Thermo Scientific,USA) at 3,000 rpm, the supernatant was used to estimate hemoglobin content employing a Thermo Spectronic Unicam UV-Vis 500 spectrophotometer (Thermo Scientific,USA) at 560 nm. The procedure was replicated thrice for each sample (n = 3), and the percentage of protection against hemolysis was determined as follows (Equation 3):

$$\% \text{ of Protection} = 100 - [(\frac{\text{Optical density of sample}}{\text{optical density of control}}) \times 100]$$

The inhibition of protein denaturation
The ability of extracts of *A. unedo* to inhibit protein denaturation was estimated according a previously described method.[21] Specifically,0.45 mL of BSA (5%, w/v) was added to 0.05 mL of extracts at various concentrations (200 to 800 µg/mL), aspirin (positive control), or distilled water (negative control).These solutions were then adjusted to pH 6.3 using a pH meter (Mettler Toledo,USA) and incubated in an Isotemp Oven incubator (Fisher Scientific,USA) at 37°C for 15 minutes, followed by 5 minutes at 70°C using an IN55m incubator (Memmert GmBH,Germany).Subsequently, 2.5 mL of PBS at pH 6.3 was added to the cooled mixture. Samples and control were then subjected to spectrophotometric analysis using a spectrophotometer (Thermo Spectronic Unicam UV-Vis 500, Thermo Scientific, USA) at 660 nm. The experiment was repeated three times and the percentage inhibition of protein denaturation was determined as follows (equation 4):

$$\% \text{ of Inhibition} = [(\frac{K0 - Kt}{K0}) \times 100]$$

Where K_0 and K_t: the absorbance values obtained respectively from the control and the plant extracts (or positive control).

In silico studies
Molecular Docking Study
To analyze the interactions of (5E)-5-Benzylidene-3-[(2-methoxyanilino)methyl]-1,3-thiazolidine-2,4-dione with α-amylase and α-glucosidase , a molecular docking study was conducted using AutoDock 1.5.6, following the protocol outlined by Trott et al. in 2010.[22] The preparation of the studied compounds and their endogenous ligand's 3D structure involved optimization using the molecular builder module in ChemDraw 23.1.1. Gasteiger partial charges were incorporated, non-polar hydrogens were merged, and rotatable bonds were defined through using the AutoDock Tools package. The high-resolution crystal structure of α-amylase (PDB ID: 4GQR; resolution 1.2 Å) and α-glucosidase (PDB ID: 5NN5; resolution 2.0 Å) were retrieved from the Protein Data Bank (http://www.rcsb.org/pdb) and served as the receptor model for docking simulations.[23, 24]
Redocking of the endogenous ligands into both α-glucosidase and α-amylase was carried out, using appropriately defined grid parameters for each enzyme. For α-glucosidase, the ligand 1- deoxynojirimycin (NOJ) was redocked into the catalytic pocket using a grid centered at coordinates x = -13.65, y = -31.49, z = 96.64, with dimensions of 40 × 40 × 40 Å. This grid encompassed the binding site of NOJ and key active site residues, specifically residues 518 and 521. Similarly, for α-amylase, the endogenous ligand MYC 3,5,7-TRIHYDROXY-2-(3,4,5-TRIHYDROXYPHENYL)-4H-CHROMEN-4-ONE was redocked using a grid centered at x = 13.433, y = 15.135, z = 39.43, with dimensions of 45 × 45 × 46 Å. Re-docking of the endogenous ligands demonstrated that it binds to the active site of α-amylase and α-glucosidase with an RMSD of less than 2 Å.

Analyse ADMET
The ADMET evaluation was conducted using the ADMETLAB 3.0 platform (https://admetlab3.scbdd.com/), as previously described by Fu et al.[25]

Statistical Analysis

The results were expressed in the present study as mean values ± standard error of the mean (S.E.M), with n=6 representing the sample size, for the *in vivo* model, and n=3 for the *in vitro* study. The data were analysed using IBM SPSS statistical software (version 25). A one-way analysis of variance (ANOVA) and Tukey's post hoc test were performed for multiple comparisons. The error significance was set at 5%. The inhibitory concentration at 50% (IC_{50}) was determined using GraphPad Prism 9.

Results and Discussion

GC-MS analysis of A. unedo L. fruit and flower extracts

Ethanolic and methanolic extracts of *A. unedo* L. fruit and flower were analysed by GC-MS and results are reported in Figure 1, 2, 3 and 4 and Tables 1 and 2. In the fruit, the chemical composition of the ethanolic extract was dominated by quinic acid (33.22%), followed by 5-hydroxymethylfurfural (21.42%) and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one (3.16%) (Figure 1 and Table 1). In the methanolic extract, azulene being the main compound (19%), followed by 2,6-di-tert-butyl-4-methyl-phenol, dodecamethylcyclohexasiloxane, octadecamethylcyclononasiloxane and hexadecamethylcyclooctasiloxane, representing 6.65%, 5.79%, 4.61% and 4.11% of the total extract, respectively (Figure 2 and Table 1).

Table 1: GC-MS analysis of phytocomponents in the ethanolic (EE) and methanolic (ME) extracts of fruit

Extract	Constituents chemical	Retention time	Molecular weight	Molecular formula	Peak area %
EE	2-Imino-1,3-Thiazolidine-3-carboxamidine	11.067	144	$C_4H_8N_4S$	0.22
	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one	14.132	144	$C_6H_8O_4$	3.16
	3,5-Dihydroxy-2-methyl-4H-Pyran-4-one	15.195	142	$C_6H_6O_4$	0.67
	Azulene	15.389	128	$C_{10}H_8$	2.00
	Hydroxymethylfurfural (HMF)	16.639	126	$C_6H_6O_3$	21.42
	Dodecamethylcyclohexasiloxane	18.710	444	$C_{12}H_{36}O_6Si_6$	0.62
	3-[N'-(3H-Indol-3-ylmethylene)-hydrazino]-5-methyl-[1,2,4]triazol-4-ylamine	21.181	255	$C_{12}H_{13}N_7$	0.86
	Tetradecamethylcycloheptasiloxane	23.033	518	$C_{14}H_{42}O_7Si_7$	0.66
	2,6-Di-tert-butyl-4-methyl-phenol	23.869	220	$C_{15}H_{24}O$	1.36
	1,3,4,5-Tetrahydroxycyclohexane-1-carboxylic acid (quinic acid)	27.490	192	$C_7H_{12}O_6$	8.38
	1,3,4,5-Tetrahydroxycyclohexane-1-carboxylic acid	27.989	192	$C_7H_{12}O_6$	24.84
	n-Hexadecanoic acid (palmitic acid)	35.383	256	$C_{16}H_{32}O_2$	0.89
	(Z)-octadec-9-enoic acid (oleic acid)	35.767	282	$C_{18}H_{34}O_2$	0.25
	Hexadecamethylcyclooctasiloxane	37.720	592	$C_{16}H_{48}O_8Si_8$	0.66
	(9Z,12Z)-octadeca-9,12-dienoic acid (linoleic acid)	38.737	280	$C_{18}H_{32}O_2$	1.09
	(Z)-octadec-9-enoic acid	39.325	282	$C_{18}H_{34}O_2$	0.50
	Octadecamethylcyclononasiloxane	40.271	666	$C_{18}H_{54}O_9Si_9$	0.81
	Tetracosamethylcyclododecasiloxane	48.687	888	$C_{24}H_{72}O_{12}Si_{12}$	0.40
ME	Azulene	15.372	128	$C_{10}H_8$	19.01
	(5E)-5-Benzylidene-3-[(2-methoxyanilino)methyl]-1,3-thiazolidine-2,4-dione	15.577	340	$C_{18}H_{16}N_2O_3S$	0.49
	Dodecamethylcyclohexasiloxane	18.701	444	$C_{12}H_{36}O_6Si_6$	5.79
	Tetradecamethylcycloheptasiloxane	23.034	518	$C_{14}H_{42}O_7Si_7$	3.99
	2,6-Di-tert-butyl-4-methyl-phenol	23.869	220	$C_{15}H_{24}O$	6.65
	Ethyl iso-allocholate	24.998	436	$C_{26}H_{44}O_5$	0.59
	Hexadecamethylcyclooctasiloxane	27.506	592	$C_{16}H_{48}O_8Si_8$	2.44
	Octadecamethylcyclononasiloxane	31.537	666	$C_{18}H_{54}O_9Si_9$	2.09
	7,9-Di-tert-butyl-1-oxaspiro[4,5]deca-6,9-diene-2,8-dione	34.202	276	$C_{17}H_{24}O_3$	0.50
	Triacontanoic acid, methyl ester	34.719	466	$C_{31}H_{62}O_2$	0.52
	Eicosamethylcyclodecasiloxane	34.862	740	$C_{20}H_{60}O_{10}Si_{10}$	1.62
	Hexadecamethylcyclooctasiloxane	37.762	592	$C_{16}H_{48}O_8Si_8$	1.67
	Octadecamethylcyclononasiloxane	40.319	666	$C_{18}H_{54}O_9Si_9$	1.50
	Octadecamethylcyclononasiloxane	42.647	666	$C_{18}H_{54}O_9Si_9$	1.02
	Tetracosamethylcyclododecasiloxane	46.847	888	$C_{24}H_{72}O_{12}Si_{12}$	1.00

In the flower, the most abundant components detected in the ethanolic extract were 5-hydroxymethylfurfural (8.51%), quinic acid (8.17%), azulene (3.36%) and benzyl.beta.-d-glucoside (2.48%) (Figure 3 and Table 2). In the methanolic extract, 5-hydroxymethylfurfural and quinic acid prevail and represent 8.92% and 6.73% of the extract, respectively, followed by azulene (2.81%) and benzyl.beta.-d-glucoside (2.46%) (Figure 4 and Table 2).In addition , to our knowledge, chemical analysis has revealed the presence of 17 new compounds in the fruits and flowers, most of which are known to have interesting therapeutic properties.

Table 2: GC-MS analysis of phytocomponents in the ethanolic (EE) and methanolic (ME) extracts of flower

Extract	Constituents chemical	Retention time	Molecular weight	Molecular formula	Peak area %
EE	1,3-Thiazolidine-2-carboxylic acid	5.205	133	$C_4H_7NO_2S$	0.27
	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	14.061	144	$C_6H_8O_4$	1.37
	Azulene	15.386	128	$C_{10}H_8$	3.36
	Hydroxymethylfurfural	16.471	126	$C_6H_6O_3$	8.51
	Dodecanoic acid (lauric acid)	19.938	200	$C_{12}H_{24}O_2$	0.24
	(3E)-5-Hydroxy-2-methyl-3-hexenoic acid	20.822	144	$C_7H_{12}O_3$	0.57
	3-Hydroxybenzoic acid	23.264	138	$C_7H_6O_3$	0.84
	2,6-Di-tert-butyl-4-methyl-phenol	23.862	220	$C_{15}H_{24}O$	1.52
	1,3,4,5-Tetrahydroxycyclohexanecarboxylic acid (quinic acid)	27.217	192	$C_7H_{12}O_6$	8.17
	Ethyl Gallate	34.850	198	$C_9H_{10}O_5$	1.72
	n-Hexadecanoic acid (palmitic acid)	35.381	256	$C_{16}H_{32}O_2$	2.19
	Benzyl. beta.-d-glucoside	40.703	270	$C_{13}H_{18}O_6$	2.48
ME	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one	14.092	144	$C_6H_8O_4$	2.34
	3,5-Dihydroxy-2-methyl-4H-Pyran-4-one	15.191	142	$C_6H_6O_4$	0.50
	Azulene	15.389	128	$C_{10}H_8$	2.81
	5-Hydroxymethylfurfural	16.518	126	$C_6H_6O_3$	8.92
	Dodecamethylcyclohexasiloxane	18.712	444	$C_{12}H_{36}O_6Si_6$	0.92
	Tetradecamethylcycloheptasiloxane	23.033	518	$C_{14}H_{42}O_7Si_7$	0.52
	2,6-Di-tert-butyl-4-methyl-phenol	23.869	220	$C_{15}H_{24}O$	1.29
	1,3,4,5-Tetrahydroxycyclohexanecarboxylic acid	27.380	192	$C_7H_{12}O_6$	3.64
	1,3,4,5-Tetrahydroxycyclohexanecarboxylic acid	27.493	192	$C_7H_{12}O_6$	3.09
	Octadecamethylcyclononasiloxane	31.506	666	$C_{18}H_{54}O_9Si_9$	0.87
	Eicosamethylcyclodecasiloxane	34.830	741	$C_{20}H_{60}O_{10}Si_{10}$	0.33
	n-Hexadecanoic acid (palmitic acid)	35.777	256	$C_{16}H_{32}O_2$	0.82
	Hexadecamethylcyclooctasiloxane	37.720	592	$C_{16}H_{48}O_8Si_8$	0.38
	Octadecamethylcyclononasiloxane	40.270	666	$C_{18}H_{54}O_9Si_9$	0.33
	Benzyl. beta.-d-glucoside	40.737	270	$C_{13}H_{18}O_6$	2.46

Anti-hyperglycemic effects analysis

Inhibitory activity of fruit and flower extracts on α-amylase and α-glucosidase enzymes, in vitro

In this assay, various fruit and flower extracts were examined for their inhibitory effect on α-amylase and α-glucosidase at different concentrations in comparison with acarbose, used as a reference drug (Figure 5). All extracts had a significant effect ($p<0.001$) on α-amylase, with an inhibitory effect enhanced by the 50% ethanolic extract of fruit with IC_{50} value of 0.31 ± 0.010 mg/mL (Table 3) in comparison with the acarbose with IC_{50} value from 0.46 ± 0.005 mg/mL, used as a control. As follows, 50% 2-propanol extract of fruit has also a high inhibitory activity with IC_{50} value of 0.46 ± 0.004 mg/mL, equivalent to the IC_{50} obtained with acarbose. In addition, the 50% 2-propanol extract of flowers showed significant inhibitory activity with an IC_{50} of 0.49 ± 0.0008 mg/mL.Similar findings were observed in terms of α-glucosidase inhibitor activity (Figure 6). The highest inhibitor activity was exhibited by 50% methanolic extract of fruit with IC_{50} value of 0.079 ± 0.001 mg/mL.Following closely by the 50% ethanolic extract of flower displaying an inhibitor activity with an IC_{50} value of 0.086±0.002 mg/mL. For instance, acarbose inhibited the α-glucosidase activity with IC_{50} value from 0.052 ± 0.0002 mg/mL.

Inhibitory activity of the extracts on α-amylase and α-glucosidase, in vivo

Following the administration of starch (2 g/kg), glucose levels rose to 1.6 ± 0.03 g/L at 60 minutes and 1.4 ± 0.01 g/L at 120 minutes, as depicted in Figure 7A. Upon the administration of 50% ethanolic extract of the fruit (150 mg/mL), a significant decrease in postprandial glycaemia was observed at both 60 and 120 minutes ($p < 0.001$). The recorded values were 1.2 ± 0.08 g/l and 0.7 ± 0.04 g/L, respectively, compared to the control group.

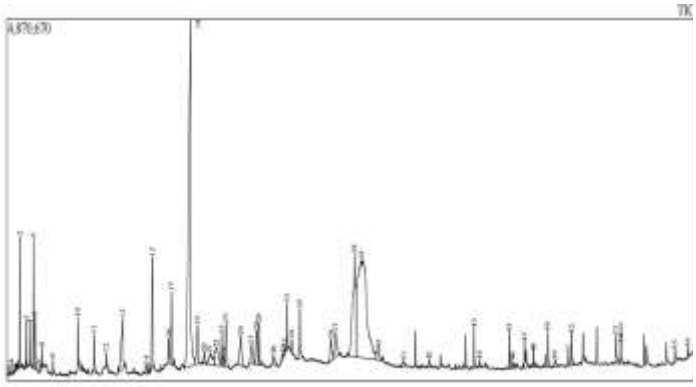

Figure 1: GC-MS chromatogram of the ethanolic extract of the fruit

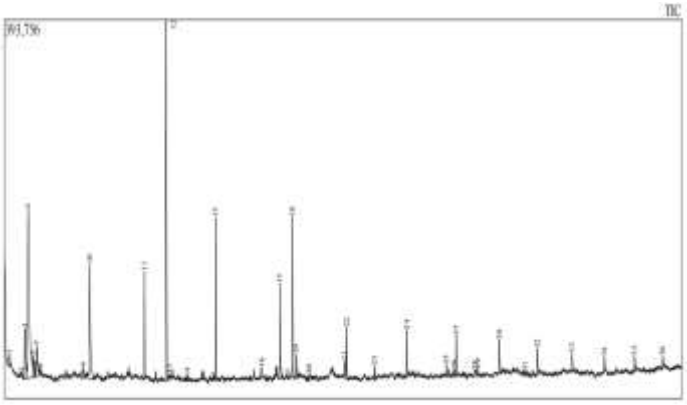

Figure 2: GC-MS chromatogram of the methanolic extract of the fruit

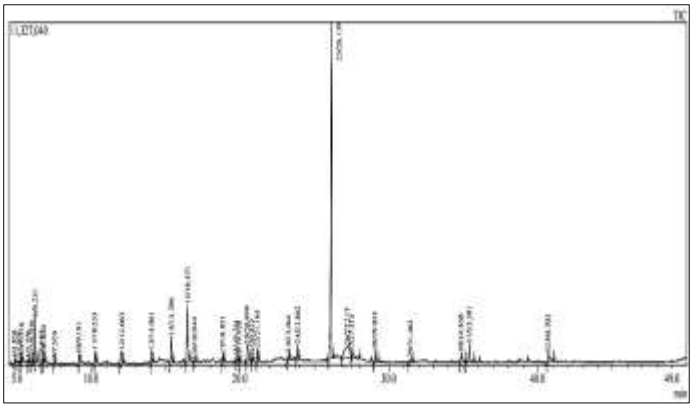

Figure 3: GC-MS chromatogram of the ethanolic extract of the flower

Likewise, in the presence of 50% 2-propanol of fruit (150 mg/mL), there was a substantial reduction in postprandial glycaemia ($p < 0.001$) at the both 60 and 120 minutes. The respective values reached were 1.15 ± 0.08 g/L and 0.8 ± 0.04 g/L, contrasting with those of the control group. Furthermore, the introduction of acarbose (10 mg/kg) markedly impeded starch-induced hyperglycemia at both 60 and 120 minutes yielding values of 0.8 ± 0.06 g/L and 0.90 ± 0.06 g/L, respectively ($p<0.001$). After the administration of sucrose (2 g/kg), glycaemia increased to 1.55 ± 0.03 g/L at 60 min and 1.38 ± 0.01 g/L at 120 min, as shown in Figure 7B. In the presence of 50% ethanolic extract of flower (150 mg/kg), postprandial glycaemia significantly decreased ($p< 0.001$) at 60 and 120 min, reaching values of 1.03 ± 0.08 g/L and 1.03 ± 0.04 g/L, respectively, compared to the control group. Likewise, with the presence of 50% methanolic extract of fruit (150 mg/kg), postprandial glycaemia significantly decreased ($p<0.001$) at 60 and 120 min, reaching values of 0.86 ± 0.08 g/L and 0.89 ± 0.04 g/L, respectively, compared to the control group. Moreover, administration of acarbose (10 mg/kg) significantly ($p<0.001$) inhibited sucrose-induced hyperglycemia at 60 and 120 minutes, giving values of 0.97 ± 0.06 g/L and 0.90 ± 0.06 g/L, respectively.

Anti-inflammatory activity of fruit and flower extracts

In this study, the anti-inflammatory potential of *A. unedo* extracts was assessed by investigating the membrane stabilization activity by red blood cells (RBCs) and inhibition of protein denaturation using bovine serum albumin (BSA). Overall, fruit had a greater effect than the flower against. The aqueous and ethanolic extracts of fruit showed a high significant protective effect, with a maximum stabilization value of 68.42 ± 0.21%, and 66.47 ± 0.16 %, respectively , and close to that obtained with aspirin (79.93± 0.58%), used as a positive control (Figure 8). Similar findings were observed regarding the inhibitory power of protein denaturation (Figure 9). The aqueous and ethanolic extracts of the fruit had reached the greatest percentages of inhibition with values of 66.87 ± 0.17 % and 62.19 ± 0.15 %, respectively. Furthermore, the most potent extracts for the flower was the aqueous extract with 57.17±0.17 %. Of particular interest, the inhibitory percentage obtained with the aspirin was 79.08 ± 0.67 %. However, the anti-inflammatory potentials of flower and fruit extracts was statistically significant ($p<0.001$) when compared with aspirin used as an anti-inflammatory reference.

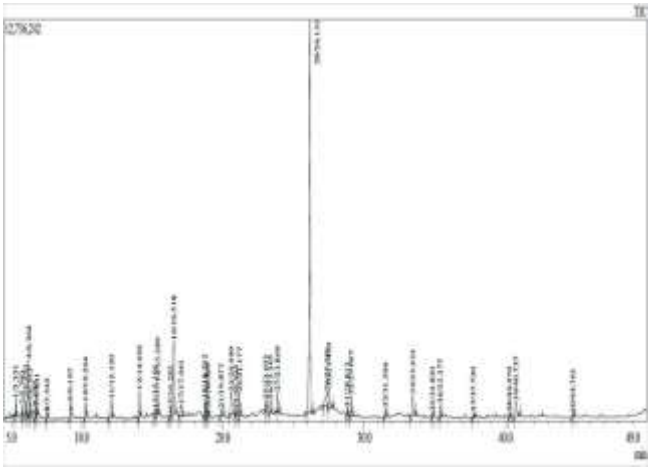

Figure 4: GC-MS chromatogram of the methanolic extract of the flower

Molecular docking study and ADMET proprieties for anti-hyperglycemic activity

The present study highlights the presence of a new compound (5(E)-5-Benzylidene-3-[(2-methoxyanilino)methyl]-1,3-thiazolidine-2,4-dione) with a promising activity that merit further analyses. Accordingly, molecular docking was performed to evaluate the binding modes of the identified compound against the α-amylase and α-glucosidase enzymes (Table 4).The docking analysis of (5E)-5-benzylidene-3-[(2-methoxyanilino)methyl]-1,3-thiazolidine-2,4-dione with α-amylase demonstrates its strong potential as an inhibitor through a well-defined network of interactions.Key hydrogen bonds with critical residues such as ARG398 and PRO332, at distances of 2.988 Å and 2.218 Å, respectively, highlight the compound's strong affinity and precise orientation within the enzyme's active site. Electrostatic interactions, particularly the pi-anion interaction with ASP402, further stabilize the binding and enhance ligand-enzyme compatibility. Additionally, hydrophobic contacts with residues like PRO4, as well as interactions involving the ligand's aromatic regions, contribute to the stabilization of the compound within the enzyme's hydrophobic pockets (Figure 10). For α-glucosidase, the molecule displays multiple significant interactions with key residues in the α-glucosidase active site, which likely interfere with substrate binding and catalysis. Key

hydrogen bonds with ARG189, SER566, THR567, and ASN570, along with a pi-anion interaction with ARG189 and a carbon-hydrogen bond with ASP243, contribute to its good binding affinity.

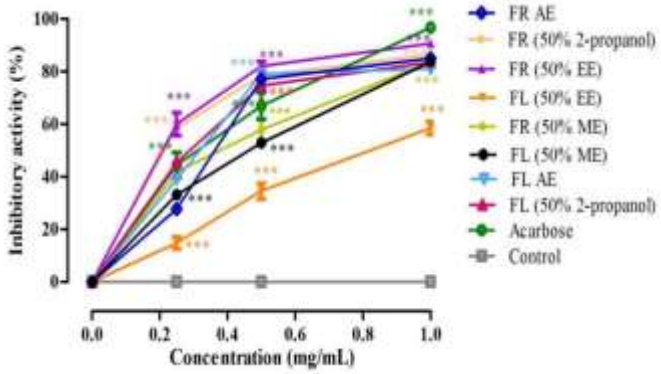

Figure 5: *In vitro* inhibitory effect of *Arbutus unedo* extracts on α-amylase enzyme activity.Values are the means ± SEM (n = 3). ***p < 0.001 as function of the control group. Fr: Fruit; FL: Flower; EE: ethanolic extract; ME: methanolic extract; AE: aqueous extract.

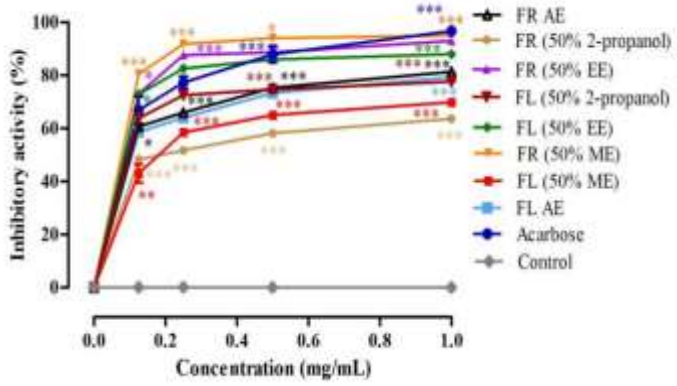

Figure 6: *In vitro* inhibitory effect of *Arbutus unedo* extracts on α-glucosidase enzyme activity.Values are the means ± SEM (n = 3). * $p < 0.05$, *** $p < 0.001$ as function of the control group. FR: Fruit; FL: Flower. EE: Ethanolic extract; ME: Methanolic extract; AE: Aqueous extract.

This synergy of hydrogen bonding, electrostatic forces, and hydrophobic interactions underpins the compound's ability to effectively inhibit α-amylase and α-glucosidase activity, thereby reducing starch hydrolysis and potentially reducing hyperglycemia (Figure 11). These results underscore the compound's therapeutic promise and provide valuable insights for future optimization in the development of novel anti-hyperglycemic agents. For ADMET analysis, 5(E)-5-Benzylidene-3-[(2methoxyanilino)methyl]-1,3-thiazolidine-2,4-dione exhibits promising drug-like properties, and it complies with Lipinski's Rule and satisfies both the Pfizer and GSK rules, suggesting favorable oral bioavailability and ADMET profiles. The compound has a molecular weight of 340.09, within the optimal range for drug-like molecules, with balanced hydrogen bond acceptors and donors (5 and 1, respectively) and low flexibility (nRot = 2). The TPSA value of 58.64 indicates good oral absorption and permeability. Its logP value (2.742) ensures favorable lipophilicity.

Traditional plant-based medicines have proved remarkably effective in the treatment of diabetes .[26] *Arbutus unedo* is one of the medicinal plants traditionally used in Morocco to treat diabetes.[14] The purpose of this work was to unveil the pharmacological potential of Moroccan *A.unedo* fruit and flower extracts, with particular emphasis on inhibition of pancreatic α-amylase and intestinal α-glucosidase, stabilization of the erythrocyte membrane and inhibition of protein denaturation. The finding of this investigation displayed that the fruit of *Arbutus unedo* has a higher quinic acid and azulene content than the flower. The ethanolic fruit extract had significant inhibition of α-amylase activity *in vitro* and *in vivo* and greater effects to those of flower and acarbose. This inhibitory power of this extracts is probably due to the presence of phenolic compounds. In fact, quinic acid belongs to the family of polyphenols, a major component of this extract (33.22%), is known for its strong inhibition of α-amylase .[27]

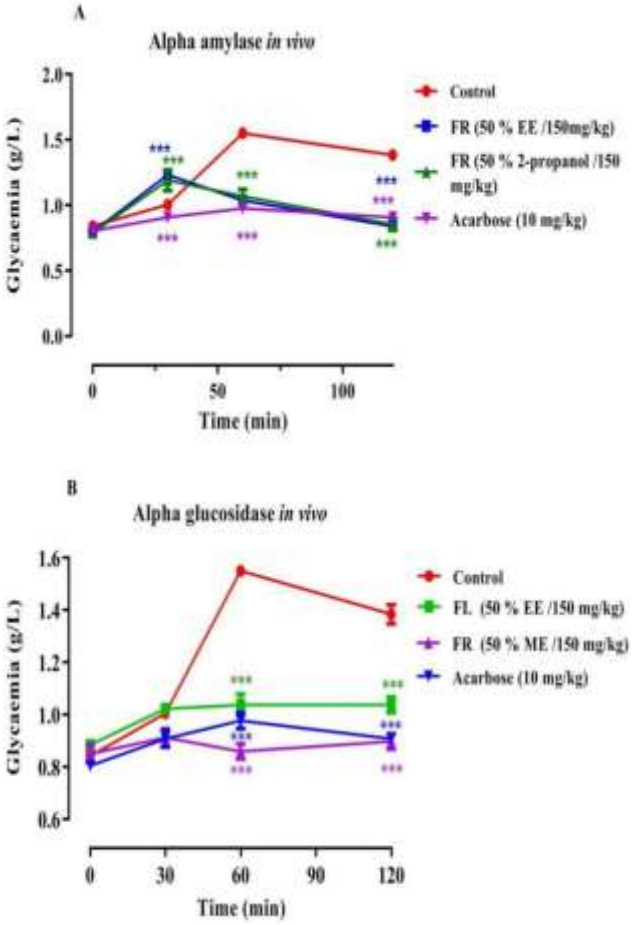

Figure 7: Effect of *Arbutus unedo* extracts (150 mL/Kg) on plasma glucose levels after starch intake (A) and sucrose intake (B) in rats.Values are the means ± SEM (n = 6). ***p <0.001 as function of the control group. FL:Flower ; FR:Fruit ; EE: Ethanolic extract ; ME: Methanolic extract.

In comparison, with previous studies, it was observed that IC50 of ethanolic fruit extract of *A. unedo* from El Harcha was lower in comparison with acarbose than dried *A. unedo* fruits and leaves extracts reported by Tenuta et al.in 2020 and Doudach et al.in 2023 (IC50 ranging from 0.078 ± 1.08 to 0.593 ± 4.57 mg/mL).[15,28] However, this variability is probably due to various factors such as extraction methods, vegetal material and environmental factors. This work displayed also that methanolic fruit extract has a powerful hypoglycemic effect by inhibiting α-glucosidase activity, and that this potential is similar to that of acarbose, a widely used α-glucosidase inhibitor.This potential is probably due to the presence of some bioactive components.Chemical

analysis have shown the presence of 5(E)-5-Benzylidene-3-[(2-methoxyanilino)methyl]-1,3-thiazolidine-2,4-dione, belonging to the thiazolidinedione family (TZDs), considered as an important class of insulin sensitizers. Of particular interest, TZDs are full PPAR-γ agonists and potent insulin sensitizers.

Table 3: IC_{50} values of extracts of *A. unedo* and acarbose on α-amylase and α-glucosidase inhibition

Plant	Extraction solvent	IC_{50} (mg/mL)	
		α-amylase	α-glucosidase
Fruit	EtOH:water (50/50) (v/v)	0.31 ± 0.010 [a]	0.088 ± 0.0001 [a]
	2-propanol: water (50/50) (v/v)	0.46 ± 0.004 [b]	0.197 ± 0.012 [a]
	Aqueous extract	0.50 ± 0.0002 [a]	0.129 ± 0.003 [a]
	MeOH:water (50/50) (v/v)	0.53 ± 0.013 [b]	0.079 ± 0.001 [a]
Flower	EtOH:water (50/50) (v/v)	0.82 ± 0.029 [a]	0.086 ± 0.002 [a]
	2-propanol : water (50/50) (v/v)	0.49 ± 0.0008	0.107 ± 0.0008 [a]
	Aqueous extract	0.50 ± 0.0001	0.180 ± 0.016 [a]
	MeOH : water (50/50) (v/v)	0.53 ± 0.011 [a]	0.098 ± 0.0008 [a]
Positive control	Acarbose	0.46 ± 0.005	0.052 ± 0.0002

Values are expressed as mean ± SEM (n=3). Means with the same exponent in the same group in the same column (a,b) are significantly different at p <0.001.

Table 4: Binding Affinity of the (5E)-5-benzylidene-3-[(2-methoxyanilino)methyl]-1,3-thiazolidine-2,4-dione with α-amylase and α-glucosidase

Compound	Target enzyme							
	α-amylase				α-glucosidase			
	Affinity (kcal/mol)	Residues	Distance (Å)	Type	Affinity (kcal/mol)	Residues	Distance (Å)	Type
(5E)-5-benzylidene-3-[(2-methoxyanilino) methyl]-1,3-thiazolidine-2,4-dione	**-6.9**	ARG398	3.55	Hydrogen Bond	**-6.5**	ARG189	2.89	Hydrogen Bond
		ARG398	2.98	Hydrogen Bond		SER566	2.92	Hydrogen Bond
		PRO332	2.21	Hydrogen Bond		THR567	3.28	Hydrogen Bond
		ASP402	3.46	Pi-Anion		ASN570	3.07	Hydrogen Bond
		SER289	3.67	Pi-Donor		SER566	2.38	Hydrogen Bond
		PRO4	4.98	Pi-Alkyl		ASP243	3.51	Carbon Hydrogen Bond
						ARG189	4.03	Pi-Anion
Acarbose	**-7.3**	ARG252	2.98	Hydrogen Bond	**-6.4**	ARG189	3.09	Hydrogen Bond
		SER289	2.95	Hydrogen Bond		TYR191	3.03	Hydrogen Bond
		ARG398	2.87876	Hydrogen Bond		ASN570	3.02	Hydrogen Bond
		ARG398	3.10	Hydrogen Bond		ASN570	2.17	Hydrogen Bond
		ARG421	3.32	Hydrogen Bond		ASP243	1.94	Hydrogen Bond
		PRO332	3.09	Hydrogen Bond		ASP243	2.48	Hydrogen Bond
		GLY334	2.22	Hydrogen Bond		SER560	2.50	Hydrogen Bond
		SER289	2.73	Hydrogen Bond		SER560	2.73	Hydrogen Bond
		TRP280	2.20	Hydrogen Bond		ARG189	3.52	Hydrogen Bond
		TRP280	2.08	Hydrogen Bond				
		HIS331	3.58	Carbon Hydrogen Bond				
		PRO332	3.39	Carbon Hydrogen Bond				

They have been used clinically to treat type 2 diabetes mellitus, but due to numerous serious side effects, these molecules were withdrawn and then reintroduced after chemical modifications.[29] Currently, growing interest is given to the chemical modification and functionalization of TZDs molecules to benefit from their anti-hyperglycemic properties with less side effects and undesirable outcomes.[30]

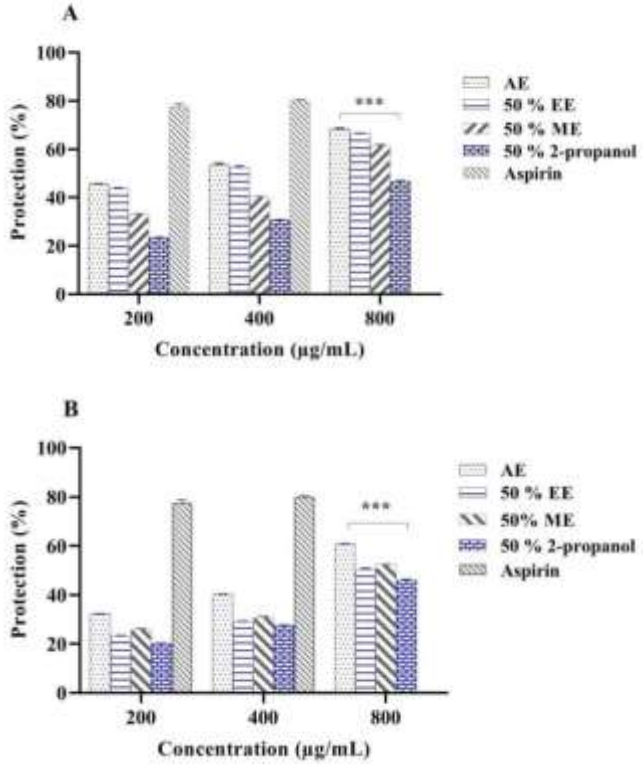

Figure 8: Effect of *Arbutus unedo* fruits (A) and flowers (B) extracts on RBC membrane stabilization.Values are presented as mean ± SEM (n = 3) ; ***$p < 0.001$ significant differences compared to the control group. ME: Methanolic extract; EE: Ethanolic extract; AE: Aqueous extract.

Recently, Patil and coll., have showed that some synthesized derivatives of 5-benzylidene-2,4-thiazolidinediones have a powerful *in vivo* hypoglycemic and lipid-lowering activities without toxic effects on the liver or heart .[31] To the best of our knowledge, the 5(E)-5-Benzylidene-3-[(2-methoxyanilino)methyl]-1,3-thiazolidine-2,4-dione found in this study is a new natural compound that had never been identified in plants before. It could be a promising molecule for further exploration of potential hypoglycemic activities to enrich and diversify the therapeutic arsenal against diabetes. In contrast, our results suggest that polyphenols extracted from the fruit of *Arbutus unedo* could reduce postprandial hyperglycemia by inhibiting activity of α-amylase. Previous research has established that flavonoids, such as anthocyanin from the fruit of *Berberis integerrima* Bunge, and polyphenols from the pericarp of *Garcinia mangostana*, have the ability to significantly inhibit α-amylase activity .[32,33] In fact, postprandial hyperglycemia can be controlled by various approaches, including inhibition of α-amylase and α-glucosidase activity.[12] Inhibitors of these digestive enzymes delay carbohydrate digestion and reduce glucose absorption. This reduction leads to a drop in postprandial glycemia, making this approach an effective therapy for type 2 diabetes mellitus.[33,34] The anti-inflammatory potential of *A. unedo* fruit extracts has been investigated in previous studies, but the flower was examined for the first time in this report. For our part, we evaluated the anti-inflammatory potential of extracts of fruit and flower of *A. unedo* El Harcha, based on erythrocyte membrane stabilization and protein denaturation inhibition. Taken together, the potential of extracts of fruit was greater than that of flowers, particularly in the aqueous extract, which could be mainly due to their bioactive compounds. These outcomes are consistent with those obtained by Lehfa et al. in 2023, who reported that the aqueous extract of the fruit had the highest protective effect (70.867 ± 0.614%).[35] These beneficial health-promoting actions could be attributed to some phyto-constituents presents in these extracts, such as azulene, 5-hydroxymethylfurfural, palmitic acid and oleic acid, which have been shown to have anti-inflammatory properties. They could also be the results of synergistic action between these products or with other components present in the extracts.[36-39] The *in vitro* erythrocyte membrane stabilization assay are commonly used methods for assessing anti-inflammatory activity. Erythrocytes undergo membrane lysis when exposed to a hypotonic environment .[9]

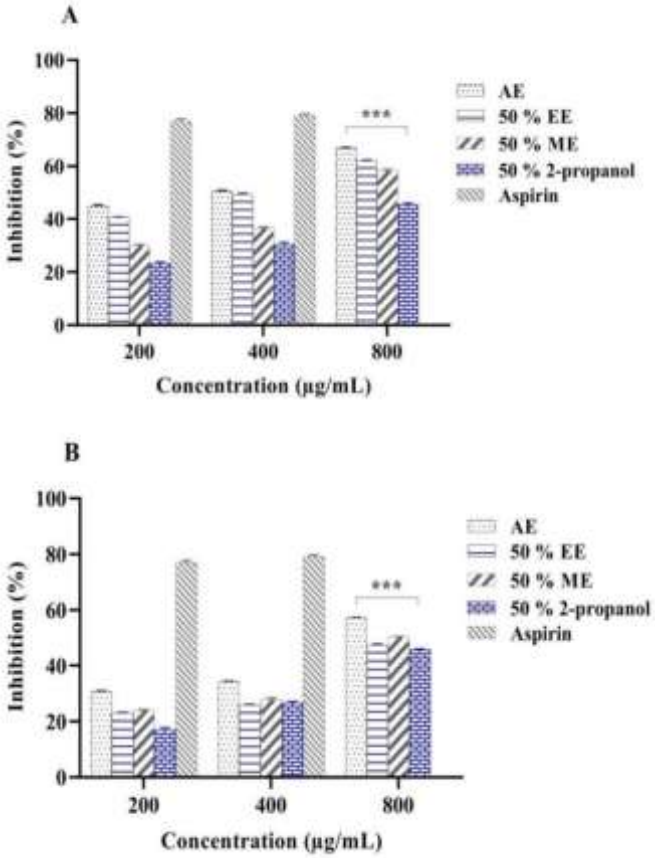

Figure 9: Effect of *Arbutus unedo* fruits (A) and flower (B) extracts on the inhibition of BSA protein denaturation.Values are presented as mean ± SEM (n = 3) ; ***$p < 0.001$ significant differences compared to the control group . ME: Methanolic extract; EE: Ethanolic extract ; AE: Aqueous extract.

It has been suggested that the agents that stabilise their membrane may also be stabilisers of the lysosomal membrane. As a result, these agents can be considered good anti-inflammatories .[10] Protein denaturation is a well-documented process, the occurrence of which is frequently attributed to inflammatory processes, especially in instances of arthritis .[40] In addition, traditional non-steroidal anti-inflammatory drugs such as indomethacin have been shown to exert their pharmacological effects via multiple mechanisms, including inhibition of protein denaturation .[9] Overall, the fruit has potential pharmacological effects and can be used to treat postprandial hyperglycemia and reduce inflammation, thereby helping to manage type 2 diabetes and prevent its complications. The present study is very informative and has many strengths: (i) the exploration of the antidiabetic status of extracts both *in vitro* and *in vivo*, (ii) the assessment of the anti-inflammatory effect of the extract, given the role of inflammation as a key factor in the development of diabetic complications, (iii) the chemical characterization of fruit and flower extracts to identify potential compounds with biological activities, and

finally (iv) the identification of a new compound with probable great hypoglycemic activity. However, the study has some limitations, including evaluation of only ethanolic and methanolic *A. unedo* extracts and the assessment of antidiabetic and anti-inflammatory effects only with crude extracts. Further in-depth research on purified compounds will provide valuable information on bioactive compounds for pharmaceutical purposes.

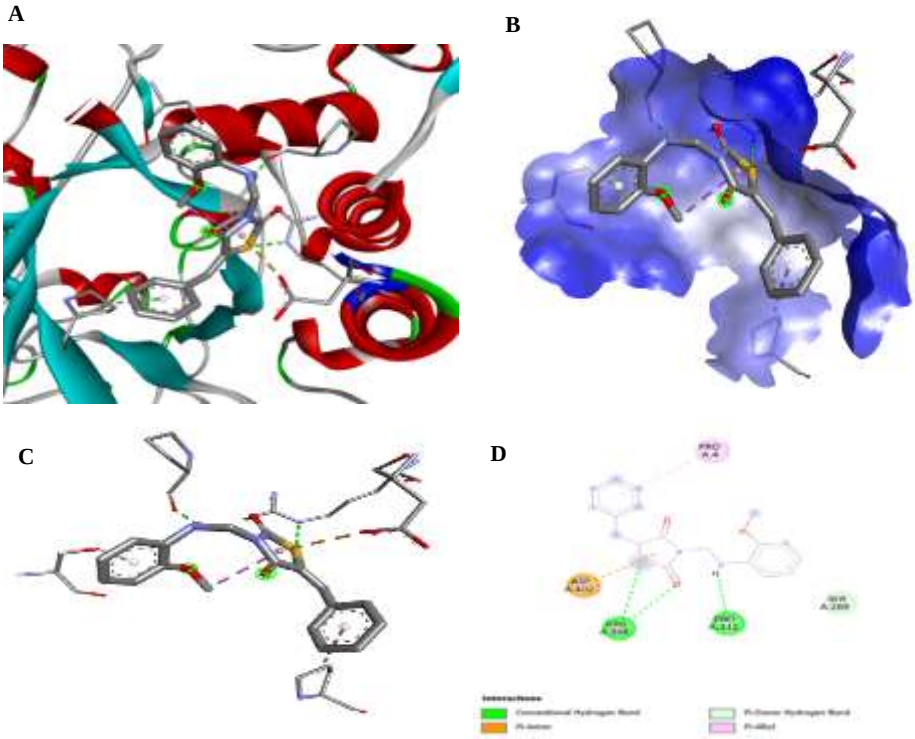

Figure 10: Molecular docking study of α-amylase.A:(5E)-5-benzylidene-3-[(2-methoxyanilino)methyl]-1,3-thiazolidine-2,4-dione and α-amylase Molecular docking, B: α-amylase hydrophobic surface, C: 3D interactions between (5E)-5-benzylidene-3-[(2-methoxyanilino)methyl]-1,3-thiazolidine-2,4-dione and α-amylase pocket, D: 2D interactions between(5E)-5-benzylidene-3-[(2-methoxyanilino)methyl]-1,3-thiazolidine-2,4-dione and α-amylase pocket.

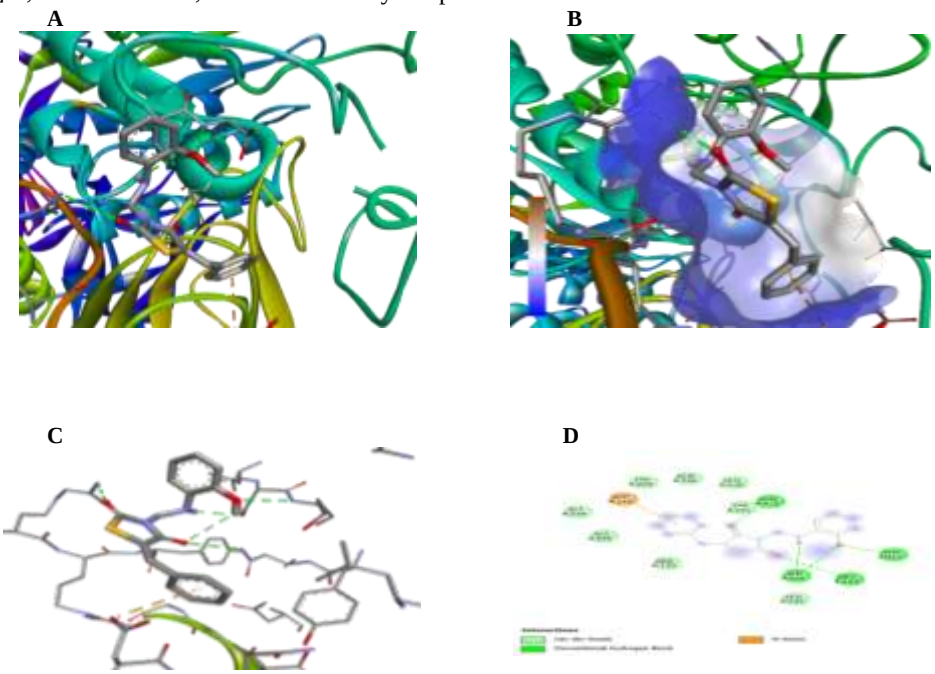

Figure 11: Molecular docking study of α-glucosidase.A:(5E)-5-benzylidene-3-[(2-methoxyanilino)methyl]-1,3-thiazolidine-2,4-dione and α-glucosidase Molecular docking. B: α-glucosidase hydrophobic surface, C: 3D interactions between (5E)-5-benzylidene-3-[(2-methoxyanilino)methyl]-1,3-thiazolidine-2,4-dione and α-glucosidase pocket, D: 2D interactions between (5E)-5-benzylidene-3-[(2-methoxyanilino)methyl]-1,3-thiazolidine-2,4-dione and α-glucosidase pocket.

Conclusion

The present study clearly displayed that fruit and flowers of *A. unedo* have significant anti-inflammatory effect and a great hypo-glycemic properties and can serve as excellent sources of natural digestive enzymes' inhibitors. Moreover, the identification of (5E)-5-benzylidene-3-[(2-methoxyanilino)methyl]-1,3-thiazolidine-2,4-dione, a new promising molecule with interesting *in silico* properties, represents a new issue for the development of natural anti-hyperglycemic drug for better management of diabetes and to improve the quality of life of diabetic patients. These results encourage further studies aimed at isolating and characterising the promising natural compounds found in this study associated with anti-hyperglycemic properties.

Conflict of interest

The author`s declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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