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Research Article

Phytochemical screening and *in vitro* evaluation of alpha amylase, alpha glucosidase and beta galactosidase inhibition by aqueous and organic *Atractylis gummifera* L. extracts

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Abstract

Diabetes is a chronic condition which is increasingly progressing throughout the world. To treat it, several methods are used, among which is medicinal plants that still have an unknown mechanism of action. The objective of this work is to evaluate the *in vitro* hypoglycemic effect of the extracts of the underground part of *Atractylis gummifera*, a member of Asteraceae used in traditional Moroccan medicine. A phytochemical study of the aqueous extracts (decocted, infused and macerated) and organic extracts (methanol, methanol macerate, chloroformic, ethyl acetate and petroleum ether), and a phytochemical screening of the different secondary metabolites was done. The antidiabetic power of the extracts of *A. gummifera* by testing the inhibitory activity of α-amylase, α-glucosidase and β-galactosidase, which are enzymes responsible for the digestion of polysaccharides was determined. The extracts of *A. gummifera* are very rich in flavonoids and tannins, and are inhibitory toα-amylase and α-glucosidase, mainly the macerate of methanol with IC_{50} values of 0.557 ± 0.013 and 0.743 ± 0.017 mg / mL respectively. Higher β-galactosidase inhibitory potential than quercetin was observed for aqueous macerates and methanol with IC_{50} values of 2.23 ± 0.012 and 2.443 ± 0.071 mg / mL respectively. The extracts of *A. gummifera* possess a significant inhibitory activity of the alpha amylase and alpha glucosidase and beta-galactosidase enzymes, in particular the macerate of methanol followed by the aqueous macerate, among the eight extracts tested.

Keywords: Diabetes; *Atractylis gummifera*; phytochemical study; α-amylase; α-glucosidase; β-galactosidase

Citation

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Introduction

Herbal medicine is important traditionally, and is widely used for the treatment of diabetes mellitus (1). Several works are in progress to reveal the secrets of medicinal plants and to study the traditional pharmacopoeia from a biological, chemical and pharmacological point of view (2).

To evaluate medicinal plants, our laboratory paid particular attention to toxic plants like *Peganum harmala*, but Lamchouri and his collaborators unveiled the pharmacological activities of this plant, mainly cytotoxicity, inhibition of the synthesis of DNA (3, 4) and antitumor activity (5, 6). The study of the toxicity of the plant allowed to determine the therapeutic

doses to be used *in vivo* to circumvent the toxicity of the plant (7). In the same vein, we have highlighted another toxic plant from the Asteraceae family, which, according to several ethno-botanical surveys, is the family of medicinal plants most widespread and most used in traditional Moroccan medicine, for the treatment of diseases of the digestive system and metabolic diseases (8, 9).

Atractylis gummifera L., one of the commonly used plants of Asteraceae, is a thorny, herbaceaous, perennial, with large roots that allowit to live for many years. It is known as *Carlina gummifera*, or a*ddad or chouk el-eulk* in Moroccan dialect. It is found in North Africa, Asia and southern Europe (Spain, Portugal, Italy, and Greece). In Morocco, the plant, which is available in all herbal medicine shops, is frequently found in the wild, but not in the desert, arid regions and the Anti-Atlas (10). This plant is widely used in traditional medicine to stop bleeding, facilitate deliveries, and treat edema, psoriasis and epilepsy (11, 12, 13). In addition, this plant has a beneficial effect on free radical scavenging and in the prevention of oxidative stress because its extracts have the ability to reduce *in vitro* the radical DPPH and inhibit the reducing power of iron (14), which are both triggers of several metabolic diseases such as diabetes mellitus (15). It is a chronic disease induced by insufficiency or resistance to insulin (16). The present study focuses on diabetes mellitus which is often treated by oral antidiabetic drugs that improve insulin sensitivity, stimulate its secretion, and/or decrease the digestion of carbohydrates by the inhibition of the enzymes responsible for transforming polysaccharides into glucose in the digestive tract (17).

A. *gummifera* is also known for its toxicity which is due to two glycosides: atractyloside and gummiferine (18). These last two glycosides inhibit oxidative phosphorylation, which prevents glucose metabolism and halts the transformation of glycogen to glucose and thus to death by hypoglycemia (0.4 g / L) (19).

The principle of toxicity of this Asteraceae and its traditional use have pushed us to use *A. gummifera* in the search for a new oral antidiabetic agent with doses much lower than the toxic dose which varies, according to Lefranc and Charnot, between 100 - 480 g fresh root infused with milk or naturally taken alone for 60 kg body weight (14). In addition, it has been shown that the intra-peritoneal lethal dose is 431 mg / kg for rats and 580 mg / kg for mice (15) and the oral lethal dose is 1100 mg / kg for rats (20).

In this regard, we conducted:

(i) A phytochemical study consisting of preparation of aqueous extracts (decocted, infused and macerated) and organic extracts (methanol, methanol macerate, chloroformic, ethyl acetate and petroleum ether),

(ii) Screening of various secondary metabolites (flavonoids, tannins, saponins, quinones, anthraquinones, anthracenosides, alkaloids and sterols), and

(iii) *In vitro* evaluation of the antidiabetic activity of the underground part of *A. gummifera* aqueous and organic extracts by inhibition of the enzymes responsible for the degradation of polysaccharides and, by implication, the increase in blood sugar, like alpha amylase which catalyzes the hydrolysis of the alpha-1,4-glucosidic bonds of starch and glycogen, alpha glucosides (maltase, lactase and saccharase) that catalyze disaccharides to monosaccharide's and beta galactosidase that hydrolyze beta-galactosides into simpler monosaccharide's (17), which are the enzymes responsible for the degradation of polysaccharides and, by implication, the increase in blood sugar.

Materials and Methods

Plant material

The present study was conducted on the underground part of A. *gummifera* which consists of roots and a long and voluminous rhizome (30 to 40 cm). The sampling was realized in the municipality of Tahla, province of Taza, which is in the region of Fes-Meknes, Morocco. It is geographically situated in a latitude of 34° 03 North, and a longitude of 4° 25 West on the main road N 6 Fez-Taza and on the highway A2.

The plant was identified using the available Floras (21, 22), by Prof. Abdeslam Ennabili and Dr. Abdelmajid Khabbach, and a voucher specimen, under the código SE01 was deposited in the Laboratory of Materials, Natural Substances, Environment and Modeling (LMSNEM), Polydisciplinary Faculty of Taza, Sidi Mohamed Ben Abdellah University of Fez, Morocco.

Preparation of plant extracts

Aqueous extracts: It is an extraction by the distilled water using three methods which vary with respect to the temperature and the time of extraction:

- Decoction: A method which consists of warming the plant parts in water that is brought to a boil for 20 min.

- Infusion: A method of extraction of active ingredients by dissolution in boiling water for 30 min.

- Maceration: A method of extraction by dissolution of the plant material in cold water for 24 hrs.

The extracts were then filtered through Whatman paper and the filtrate obtained was evaporated under reduced pressure, using a rotary evaporator, and stored at 4˚C.

Organic extracts: The organic extracts were prepared by two separation technique to extract the compounds of this plant. The first technique is realized under cold conditions by methanol maceration of 48 hrs, and the second technique is realized in high temperature using a Soxlhet, where 20 g of plant material are introduced into a cartridge of cellulose attached to a ball and surmounted by a cooler. 200 mL of different solvents with a decreasing polarity (methanol, chloroform, ethyl acetate and petroleum ether) are vaporized and then condensed with the dry powder of plant material. The extraction is ended when the solvent of extraction becomes clearer, six hrs for our experimental conditions.

Phytochemical Screening

The phytochemical screening allows to define the presence or absence of secondary metabolites (tannins, anthraquinones, flavonoids, saponins, alkaloids, quinones, sterols and anthracenosides) in the plant first and then in all the extracts from the plant.

Test for the detection of Tannins:

- Plant: 1.5 g of the dry underground parts are homogenized with 10 mL of methanol (90 %) and submitted to an agitation of 15 min. The obtained solution was filtered then we added some drops of chlorure ferrique $FeCl_3$ (1 %). The color turned to dark blue in the presence of Gallic tannins and to the greenish brown in the presence of catechic tannins (23).

- Extracts: For the characterization of tannins, we added a few drops of $FeCl_3$ (2 %) to 1 mL of each of our eight aqueous and organic extracts prepared from the underground part of *A. gummifera* dissolved in 10 mL of distilled water. The color turned to dark blue in the presence of Gallic tannins and to the greenish brown in the presence of catechic tannins (23).

Test for the detection of Anthraquinones:

- Plant: To verify the presence of anthraquinones in our plant, we prepared a maceration of 15 min of 2 g of the underground part of the plant in 20 mL of chloroform to which we added 1 mL of aqueous KOH (10 %). After stirring, the presence of anthraquinones is confirmed by a shift of the aqueous phase to the red (24).

Test for the detection of Flavonoids:

- Plant: To verify the presence of the flavonoids, we added 0.5 g of the underground part of the plant in 10 mL of distilled water, after a maceration of 15 min with stirring, the extract was filtered and put in a tube, then, we added 5 mL of ammonia solution (10 %), then, 1 mL of sulphyric acid was added. The final appearance of a yellow color indicated the presence of flavonoids (23).

- Extracts: The screening of flavonoids in the extracts was done by mixing 2 mg of each extract with 5 mL of concentrated HCl, and two or three piece of magnesium ribbon. The appearance of a red or orange color indicates the presence of the flavonoids (25).

Test for the detection of Saponins:

- Plant: The presence of saponines is verified by the preparation of a decoction of 2 g of the underground part of the plant with 100 mL of distilled water, from this solution, we prepared 10 tubes with 1 mL, 2 mL, 3 mL, 4 mL, 5 mL, 6 mL, 7 mL, 8 mL, 9 mL, 10 mL, we straightened the final volume in 10 mL with some distilled water. Then, the tubes were shaken while hanging them in a horizontal position for 15 sec. The presence of saponines was confirmed by the appearance of foam superior than 1 cm and persistent during 15 min (25).

- Extracts: 5 mg of each extract was diluted in 5 mL of distilled water, and shaken vigorously in a test tube for 15 sec. The appearance of foam (higher than 1 cm) which was stable and persistent for 15 min indicates the plentiful presence of saponines (25).

Test for the detection of Alkaloids*:*

- Plant: The detection of alkaloids was made by the general revelators of alkaloids: the Dragendorff reactive (iodobismuthate of potassium) and the reactive of Valser-Mayer (tétra-iodomercurate of potassium). For that reason, we prepared a maceration of 24 hrs by 10 g of the underground part of the plant and 50 mL of H_2SO_4 (10 %). The solution obtained after filtration was made up to 50 mL with distilled water. 1 mL of the filtrate was taken in two tubes to which 5 drops of the reactive Mayer or Drangendorf were added. The presence of alkaloids was confirmed by the appearance of orange-colored or brown-red precipitate- reddish by the reactive of Drangendorf and a white-yellow wish precipitated by the reactive of Mayer (23).

- Extracts: To verify the presence of alkaloids in the various extracts, 0.1 g of residue was included in 6 mL of ethanol 60% and then distributed in 2 test tubes. In the first tube, we added 2 drops of the Dragendorffre agent, the appearance of an orange-colore or reddish brown precipitate indicated the presence of alkaloids. In the second tube, 2 drops of the Mayer reagent was added; the appearance of a cream precipitate confirms the presence of alkaloids (26).

Test for the detection of Quinones:

- Plant: To verify the presence of the free quinones in the plant we prepared a maceration of 24 hrs of 1 g of the underground part of the plant in 15 mL of petroleum ether. After the filtration of the solution, we concentrated using a rotary evaporator. The presence of free quinones was confirmed when the aqueous phase transferred to the yellow, red or purple after adding some drops of NaOH (0.1 M) (27).

- Extracts: To 2 mL of hydroxide of sodium (10 M) 0.01 g of extract was added and shaken vigorously. The fast or slow appearance of an orange red tint indicated the presence of quinones (27).

Test for the detection of Sterols

- Plant: To verify the presence of sterols in the plant, we prepared a maceration of 24 hrs to 1 g of the underground part of the plant in petroleum ether. After filtration, the solvent was evaporated, and the residue was back by 1 mL of chloroform on which we added 1 mL acetic anhydride and 3 drops of sulphuric acid. The presence of sterols was confirmed by the appearance of a purple tint becoming gradually green. The green tint stabilized at the end of 30 min (23).

- Extracts: Sterols were tested by the reaction of Liebermann. 10 mg of the extract was dissolved in 1 mL of acetic anhydride, to which 0.5 mL of concentrated sulfuric acid was added. The appearance, in the interphase, of a purple ring turning to the blue then green indicated a positive reaction (23).

Test for the detection of Anthracenosides

- Plant: To verify the presence of anthracenosides in the underground part of the plant, we prepared a maceration for 15 min of 1 g of the plant in 10 mL of some chloroform with agitation and stirring, after the filtration of the solution, we added the ammonia of water half diluted, the presence of anthracenosides was confirmed by the appearance of a red tint which was developed after settling (28).

- Extracts: The screening was done by the reaction of Borntraeger, where 0.1 g extract was soaked with 5 mL of chloroform in a test tube for 15 min, with shaking every 5 min. The solution was then filtered on a filter paper, and then 2 mL of ammonia were added to it. After agitation, the appearance of a red tint indicates the presence of the anthracenosides (28).

In vitro antidiabetic activity

α-Amylase inhibitory activity

The *in vitro* antidiabetic power was realized by testing the inhibitory activity of the extracts on salivary and pancreatic α-amylase which catalyses the hydrolysis of the alpha-1,4-glycosidic bonds of polysaccharides such as starch and glycogen. This enzyme activates the first stage of the digestive process of carbohydrates, causing the increase in blood sugar (29).

To make this test, 250 µL extracts of different concentrations were mixed with 250 µL of the solution of α-amylase enzyme (240 U / mL) and incubated at 37°C for 20 min. Then, 250 µL of starch (1 %) was added, and the mixture was incubated at 37°C for 15 min. Then, 250 µL of DNS solution 40 mM (dinitro salicylic acid) which would stop the enzymatic reaction was added. The various tubes were shaken and placed in a boiling water bath for 10 min. The mixture was finally diluted with the addition of 10 mL of distilled water, and the absorbance was measured at 540 nm in a JENWAY 6305 spectrophotometer (30, 31).

The inhibition of α-amylase is expressed as percentage of inhibition calculated by the following equation: Inhibition (%) = $[(Ac - Acb) - (As - Asb) / (Ac - Acb)] \times 100$,

where Ac refers to the absorbance of control (enzyme and buffer); Acb refers to the absorbance of control blank (buffer without enzyme); As refers to the absorbance of sample (enzyme and inhibitor); and Asb is the absorbance of sample blank (inhibitor without enzyme).

α-Glucosidase Inhibitory Assay

The α-glucosidase is an enzyme secreted by the cells of the intestinal membrane, it activates the final stage of the process of the hydrolysis of disaccharides to monosaccharides, which are absorbable by the intestinal cells and which in turn causes the blood sugar levels to increase (32). The inhibition of this enzyme was done *in vitro* by mixing the enzyme with the substrate which is a bound molecule of glucose alpha-1,4 in a nitrophénol: p-nitrophényl-a-D-glucopyranoside (pNPG). In the absence of the inhibitor, alpha-glucosidase cuts the alpha-1,4 bond of the molecule of pNPG, which results in a molecule of nitrophénol and a molecule of glucose.

The hydrolysis results in a yellow tint, the intensity of which is measured using a spectrophotometer.

For testing the inhibitory effect of extracts, 150 µL of the same in various concentrations and 100 µL of the solution of the enzyme α-glucosidase (0.1 U / mL) prepared in the buffer phosphate (0.1 M) in pH = 6.7 were incubated in 37°C for 10 min. Then, 200 µL of 1mM p-nitrophényl-a-D-glucopyranoside (pNPG) solution in 0.1 M sodium phosphate buffer (pH = 6.7) was added. The mixture was incubated in 37°C during 30 min. At the end, 1 mL of Na_2CO_3 (0.1 M) was as added and the reading of the results was made in the wavelength 405 nm by means of a JENWAY 6305 spectrophotometer (32).

Standard acarbose, medicine reference, is used as a positive control. The inhibition of α-glucosidase is expressed by a percentage of inhibition and calculated by the following equation: inhibition (%) = $[(Ac - Acb) - (As - Asb)/(Ac - Acb)] \times 100$,

where Ac refers to the absorbance of control (enzyme and buffer); Acb refers to the absorbance of control blank (buffer without enzyme); As refers to the absorbance of sample (enzyme and inhibitor); and Asb is the absorbance of sample blank (inhibitor without enzyme).

Table 1: Efficiency of various phytochemical extraction procedures used in *Atractylis gummifera* L.

Extract	Extraction efficiency (%)
Decocted	35
Infused	32.5
Macerated	24.5
Methanol	15
Macerated Methanol	6.66
Chloroform	3.33
ethyl acetate	3.33
petroleum ether	2.63

Table 2: Phytochemical screening of *Atractylis gummifera* L.

Plant and Extracts	Tannins	Anthraquinones	Flavonoids	Saponines	Alkaloids	Quinones	Sterols	Anthacenosides
Plant	+++	-	+++	+	-	++	+	-
Decocted	+++	-	++	+	-	-	-	-
Infused	+++	-	+++	+	-	-	-	-
Macerated	+++	-	+++	-	-	-	-	-
Methanol	+++	-	+++	-	-	-	-	-
Macerated methanol	+++	-	+++	+	-	-	-	-
Chloroform	+++	-	+++	-	-	-	-	-
Ethyl acetate	+++	-	+++	-	-	-	-	-
Petroleum ether	+	-	+	-	-	-	-	-

(+ Presence, ++ medium presence, +++ strong presence, - Absence)

β-Galactosidase inhibitory activity

The β-galactosidase or the lactase is a glycoside hydrolase enzymes that catalyses the hydrolysis of β-galactosides into monosaccharides (33). For the inhibition of this enzyme, a mixture of 150 µL of various extract concentrations and 100 µL sodium phosphate buffer (0.1 M) in pH = 7.6 containing the solution of enzyme β-galactosidase (100 U / mL) was incubated in 37°C for 10 min. Then, added 200 µL of 2-nitrophenyl beta-D-galactopyranoside (1 mM) solubilized in sodium phosphate buffer (0.1 M) in pH = 7.6. Reaction mixtures were incubated in 37°C for 30 min. After incubation, 1 mL of Na_2CO_3 was added to stop the reaction and recorded the absorbance at 410 nm by using the JENWAY 6305 spectrophotometer (33).

Quercetin was used as positive control, and the percentage of inhibition was calculated according to the following equation: inhibition (%) = $[(Ac - Acb) - (As - Asb) / (Ac - Acb)] \times 100$,

where Ac refers to the absorbance of control (enzyme and buffer); Acb refers to the absorbance of control blank (buffer without enzyme); As refers to the absorbance of sample (enzyme and inhibitor); and Asb is the absorbance of sample blank (inhibitor without enzyme).

Statistical analysis

The inhibition activity of the three enzymes has been expressed as percentage of inhibition. The IC_{50} value was determined and compared with reference medicine. The results are expressed as averages of three determinations ± standard error mean (SEM). It was analyzed by one-way analysis of variance (one-way ANOVA) using the software GraphPad Prism.

Results

Extraction efficiency

The extraction yield is the quantity of compounds or substances which can be extracted by a solvent according to the method of extraction and was calculated by the following relation: % = Mass of extract * 100 / mass of the plant powder. The extraction yield by the various solvents is summarized in Table 1.

Phytochemical screening

The revelation of the various classes of secondary metabolites was realized at first on the powdered whole plant of *A. gummifera*. Afterward, secondary metabolites were screened in the various aqueous and organic extracts of the plant. Thus, we were able to make preliminary determination of the phytochemical composition of each extract and the results obtained are summarized in the Table 2.

In vitro antidiabetic activity

The results obtained showed that *A. gummifera* extracts possess inhibitory activity of α-amylase, α-glucosidase and β-galactosidase, and that the

Table 3: IC_{50} (µg / mL) of α-amylase, α-glucosidase and β-galactosidase inhibitory activity with aqueous and organic extract of *Atractylis gummifera* L.

Extracts	α-amylase	α-glucosidase	β-galactosidase
Methanol	924 ± 0.067[d]	1236 ± 0.089[d]	4558 ± 0.052[g]
Macerated methanol	**557 ± 0.013**[b]	**743 ± 0.017**[b]	**2443 ± 0.071**[b]
Chloroform	1256 ± 0.029[f]	1674 ± 0.039[h]	3300 ± 0.068[d]
Ethyl acetate	1397 ± 0.010[g]	1863 ± 0.013[g]	2549 ± 0.204[c]
Petroleum ether	1605 ± 0.005[h]	1509 ± 0.011[f]	4440 ± 0.131[f]
Decocted	1352 ± 0.060[g]	1802 ± 0.080[g]	4337 ± 0.160[e]
Infused	852 ± 0.128[c]	1133 ± 0.171[c]	3239 ± 0.163[d]
Aqueous macerated	1000 ± 0.055[e]	1461 ± 0.047[e]	**2230 ± 0.012**[a]
Acarbose	**311 ± 1.380**[a]	**1801 ± 2.000**[a]	-
Quercetin	-	-	**2460 ± 1.09**[b]

The values are the mean of three determinations ± standard error.
Values in the same column not sharing a common letter (a to h) differ significantly at $p<0.05$

percentage inhibition of these enzymes increases according to the concentration of the extracts and reaches a saturation level at a well-defined concentration for each enzyme.

The α-amylase inhibitory activity of *A. gummifera* extracts is shown in Fig. 1, where all extracts inhibit α-amylase activity at concentrations ranging from 500 to 2500 µg / mL. In addition, all extracts showed a statistically significant difference ($p<0.05$) with respect to the reference compound, acarbose. The methanol macerate extract showed the strongest inhibitory activity of α-amylase with an IC_{50} value equal to 557 ± 0.013 µg / mL (Table 3) and with a statistically significant difference compared to the other extracts ($p<0.05$). This result remains lower than the inhibitory activity of acarbose (IC_{50} = 311 ± 1.38 µg / mL), which is a pure molecule used for the treatment of diabetes mellitus, but which triggers a lot of side effects and has a lot of contradictions.

In the same way, extracts of *A. gummifera* (666 - 3330 µg / mL) showed an inhibitory effect on α-glucosidase which depends on the concentration tested (Fig. 2). The inhibitory activity increases in parallel with the increase in the concentration of the extracts. All the extracts tested had an inhibitory effect of α-glucosidase with a better activity noted in the methanol macerate (IC_{50} = 743 ± 0.017 µg / mL), but this result remains lower than that of the reference medicine, acarbose (IC_{50} = 180.1 ± 2.00 µg / mL) (Table 3) with statistically significant differences ($p<0.05$).

Similarly, the inhibitory activity of β-galactosidases which hydrolyzes beta-galactosides was also blocked by extracts of *A. gummifera* at varying concentrations ranging from 666 to 5000 µg / mL, where the percentage of inhibition increases in accordance with the increase in concentration levels (Fig. 3). The aqueous and methanol macerates have the best inhibitory activity of this enzyme with IC_{50} values of 2230 ± 0.012 and 2443 ± 0.071 µg / mL respectively (Table 3). Statistically, there are insignificant differences between these two extracts and also insignificant differences with regard to the reference product; quercetin, which has an IC_{50} value equal to 2460 ± 1.09 µg / mL. However the differences are statistically significant compared to the other extracts of the plant.

Discussion

The extraction yield of the underground part of *Atractylis gummifera* varies according to the extraction method (hot or cold) and depending on the solvent used during the extraction. In fact, it is noted that the best yields are obtained with the most polar solvents (aqueous extracts > methanol > chloroform > ethyl acetate > petroleum ether). Similarly, hot extraction gives the best yields compared to cold extractions for aqueous and organic extractions. The yields of the aqueous extracts are 35 % for the decoction and 24.5 % for the macerated. On the other hand, for organic extracts, the hot extraction with methanol makes it possible to obtain 15 % yield compared to 6.66 % in cold.

In the course of phytochemical screening, we found that the underground part of *A. gummifera* is rich in secondary metabolites that has interesting biological activities. As a matter of fact, the powder of the plant is rich in tannins, flavonoids, and contains traces of saponins, quinones, and sterols. Likewise, the aqueous extracts (decocted, infused and macerated) and organic extracts (methanol, macerated with methanol, chloroform and ethyl acetate) are very rich in tannins, flavonoids with the exception of the petroleum ether extract which has only traces of flavonoids and tannins. *A. gummifera* extracts do not contain anthraquinones, alkaloids and anthracenosides. From these results, we can conclude that the toxicity of *A. gummifera* is not due to the alkaloids but rather to two glycosides which are: the atractyloside discovered by Lefranc in 1868 (34) and gummiferine, which is a derivative of atractyloside but of much greater toxicity, which was discovered by Daniali *et al*. In 1964 (35). Atractyloside and other toxic glycosides are naturally found in plants and the levels vary

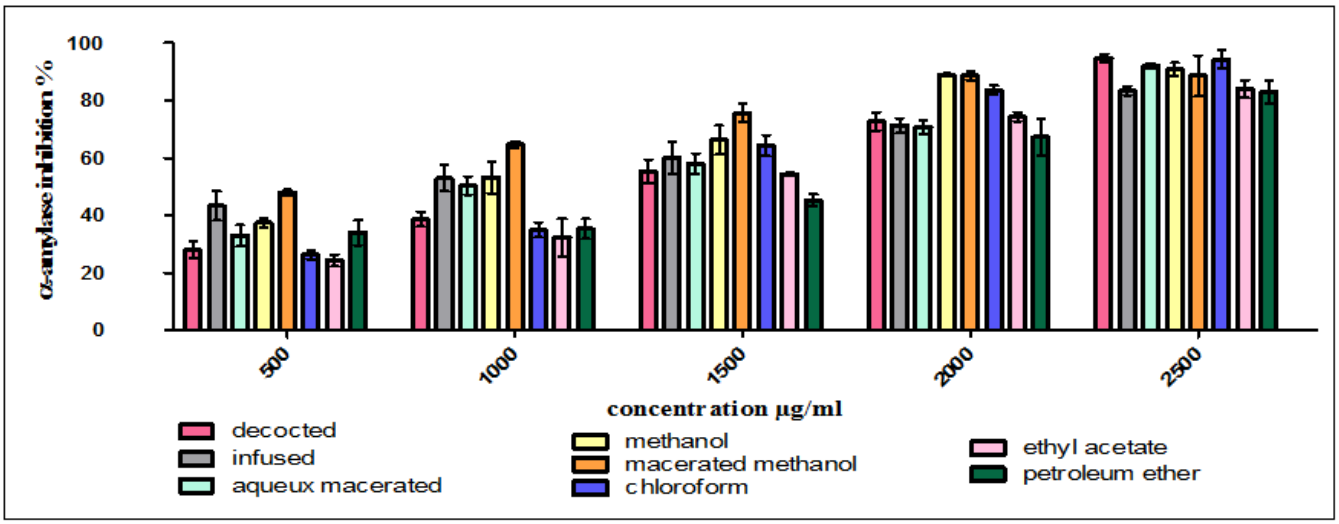

Fig. 1: Inhibition percentage of α-amylase by aqueous and organic extracts of *Atractylis gummifera* L.

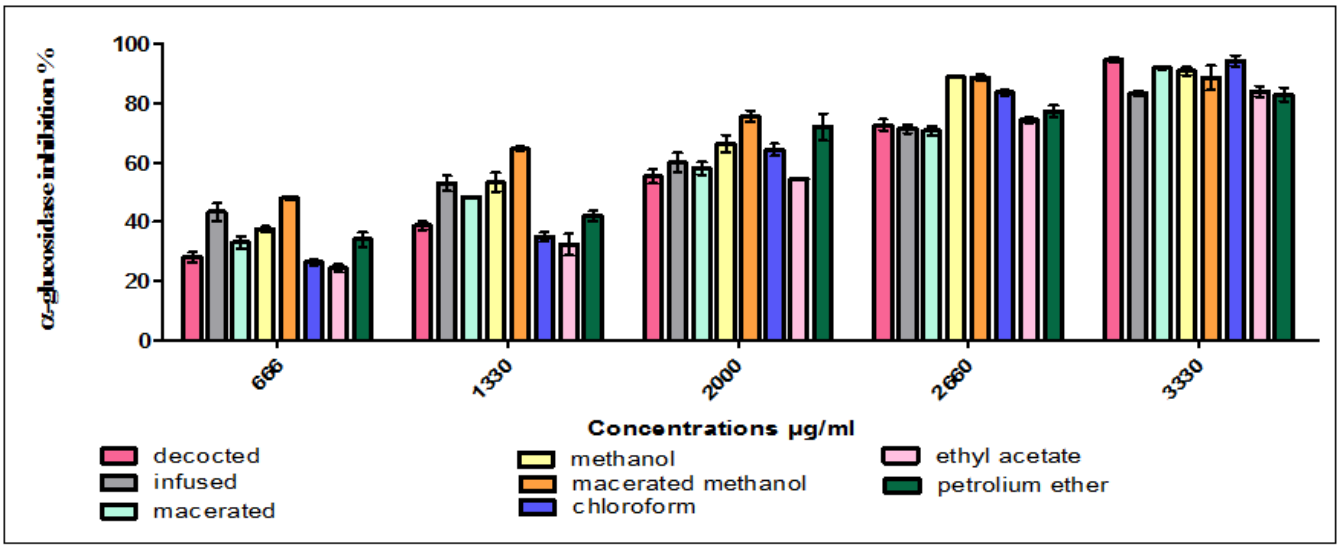

Fig. 2: Inhibition percentage of α-glucosidase by aqueous and organic extracts of *Atractylis gummifera* L.

from one species to another and even in the same species depending on the climatic conditions and the nature of the soil (20). Although these glycosides are well described from a chemical, biochemical and toxicological point of view, their metabolisms and pharmacokinetics have not yet been well explained. Likewise, *Coffea arabica* bean, which is consumed by humans, is considered among the plants that are very rich in atractyloside (36, 37).

Regarding the inhibitory activity of the extracts on the three enzymes, α-amylase, α-glucosidase and β-galactosidase, it was observed to be higher with cold-processed *A. gummifera* extracts and the more polar solvents; namely, aqueous and methanolic macerates, which may therefore be a promising source for the reduction of postprandial glucose. This finding is consistent with previous studies which demonstrated that some medicinal plant extracts such as aqueous-ethanolic extract, expressed at 75 % of propolis, inhibits α-glucosidase with an IC_{50} of 7.24 ± 1.16 µg / mL (35). Also, the ethanolic extract of the aerial part of *Miconia* sp. has an IC_{50} for α-amylase and α-glucosidase of 28.23 ± 2.15 and 1.95 ± 0.15 µg / mL respectively (38). In addition, a study conducted in our laboratory on the aqueous extract of the aerial part of *Cistus salviifolius* showed a remarkable inhibitory power of α-amylase and the α-glucosidase with IC_{50} of 217.10 ± 0.15 and 0.95 ± 0.14 µg / mL respectively (39). The enzymes of these plants have inhibitory activities more powerful than the synthetic marketed inhibitors which are costly (64.40dh ACARBOSE LAPROPHAN 100 mg ~ 6 €) and which cause a lot of undesirable side effects.

Despite the fact that α-amylase and α-glucosidase inhibitory activity of *A. gummifera* macerates is relatively lower than the activity of reference drugs, the latter are pure molecules with many side effects. Our extracts, however, are still mixtures which probably contain several molecules that need to be purified and fractionated to isolate the active ingredient. In addition, the concentrations chosen and used in our tests are in level (between 500 and 5000 µg / mL) which is well below the toxic doses. In fact, it has been proven that the lethal dose in rats is 431

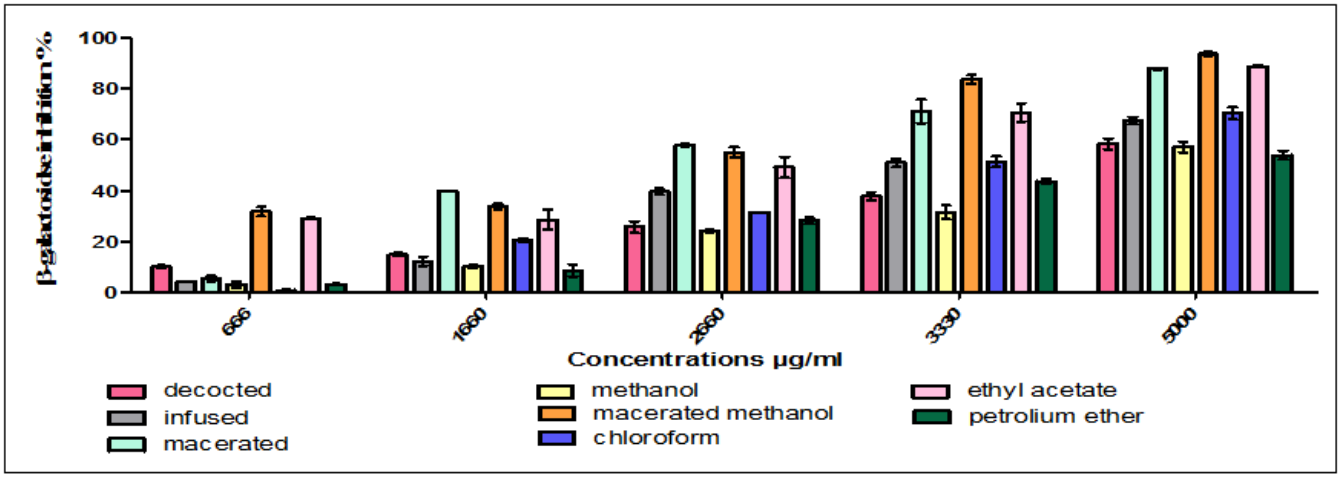

Fig. 3: Inhibition percentage of β-galactosidase by aqueous and organic extracts of *Atractylis gummifera* L.

mg / kg and in mice 580 mg / kg injected intraperitoneally (15), and 1100 mg / kg in rats when taken by mouth (20). Therefore, the doses of our study are safe. The phytochemical study indicated that aqueous and methanolic macerates are very rich in phenolic compounds (flavonoids and tannins), suggesting that the latter are potentially responsible for the inhibition activity of α-amylase, α-glucosidase and β-galactosidase. Indeed, phenolic compounds are known for their ability to inhibit carbohydrate-hydrolyzing enzymes. It has been demonstrated in the work conducted by Shobana et al. in 2009 (40) that the phenolic compounds extracted from the millet seed layer and characterized by high pressure liquid chromatography (HPLC) and electrospray ionization mass spectrometry (ESI- MS), have the highest inhibition of α-glucosidase and pancreatic amylase, with IC_{50} values of 16.9 and 23.5 µg/ mL respectively. Thus, we can deduce that aqueous and methanolic macerates of *A. gummifera* which are very rich in phenolic compounds can be a good source of therapeutic agents against type 2 diabetes; one of the diseases that is widely treated by medicinal and aromatic plants (41).

Thus, we can explain the high frequency of the use of this plant, and the Asteraceae family in general, in traditional Moroccan medicine for the treatment of several pathologies, and also justify the results of the ethnobotanical survey that was conducted among herbalists, traditional healers and druggists from the region of Meknes-Tafilalet in Morocco that showed *Atractylis gummifera* to be one of the most widely used plants in this region. It occupies the third position regarding the frequency of use for the treatment of disorders of the digestive tract and cardiovascular issues (10).

Conclusion

The current study shows that the yield and efficiency of compounds in *Atractylis gummifera* is much better with hot extraction methods and with polar solvents. The underground part of this plant is very rich in polyphenols, flavonoids and tannins, and does not contain alkaloids, anthraquinone and anthracenosides. The results of the *in vitro* antidiabetic study showed that the extracts of *A. gummifera* possess a significant inhibitory activity of the alpha-amylase and alpha-glucosidase and beta-galactosidase enzymes, especially the macerate of the methanol which has the most potent inhibitory power of α-amylase and α-glucosidase with IC_{50}s of 557 ± 0.013 and 743 ± 0.017 µg / mL respectively, followed by aqueous macerate which has the strongest inhibitory potential of β-galactosidase with IC_{50} of 2230 ± 0.012 µg / mL. These two extracts are the most active among the eight aqueous and organic extracts tested at concentrations below the toxic doses (between 430 and 580 mg / kg in animals and between 100 g and 480 g for an adult human of 60 kg). This will guide us for the exploitation and bioguided fractionation and isolation of these two extracts during further pharmacological and phytochemical investigations. In fact, an *in vivo* study is underway for the evaluation of the acute toxicity of the two extracts which have proved to be the most active *in vitro* and which will be tested *in vivo* to determine the therapeutic doses to be used for the *in vivo* study. Similarly, the *in vivo* study of the antidiabetic activity of the aforementioned extracts of *A. gummifera* is planned to start right after the end of the toxicological study.

Competing Interests

The authors have no conflict of interest.

Authors' Contribution

KB performed experimental studies, statistical analysis, and manuscript preparation. FL designed the experiments, consistent guidance, analysed the data, justified through discussion and manuscript preparation and review. HT designed the experiments, provided consistent guidance and manuscript review. KS supported the experimental works. YC offered consistent guidance.

MEA F: Designed the experiments, consistent guidance and manuscript review.

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