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Evaluation of the hypoglycemic effect of seven wild folkloric edible plants from Palestine

(Antidiabetic effect of seven plants from Palestine)

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Abstract:

Alfa-amylase inhibitors are used to reduce glucose absorption by suppressing carbohydrate digestion. The current study aimed to evaluate seven wild edible Palestinian plants' hydrophilic and lipophilic fractions against porcine pancreatic α -amylase enzyme. The lipophilic fractions of *Arum palaestinum*, *Malva sylvestris*, *Plantago major*, *Centaurea iberica*, *Cichorium endivia*, *Bituminaria bituminosa*, *Sisymbrium irio* leaves were sequentially separated with a nonpolar solvent hexane, while the hydrophilic fractions of the studied plants were separated with polar solvents ethanol and water. The activity of α -amylase inhibition was carried out by using α -amylase porcine pancreatic enzyme and 3,5-dinitrosalicylic acid (DNSA) method as well as by using Acarbose as a positive control. Among the studied plant's hydrophilic fractions, *C. iberica* and *C. endivia* have the highest porcine pancreatic α -amylase inhibitory effect with an IC_{50} value of 12.33 μ g/mL and 9.96 μ g/mL, respectively. In addition, among the studied plant's lipophilic fractions, *S. irio* and *A. palaestinum* have the highest porcine pancreatic α -amylase inhibitory effect with an IC_{50} value of 7.72 μ g/mL and 25.3 μ g/mL, respectively. In fact, these revealed results were near the values of Acarbose. The hydrophilic fractions of *M. sylvestris* and the lipophilic fractions of *P. major* plants exhibit remarkable α -amylase inhibitory activity. Hence, these leaves have a potential for use as regular supplements also; further investigations are required to isolate pure pharmacological molecules and to design suitable pharmaceutical dosage forms with anti-diabetic activity.

Keywords: hydrophilic fraction, lipophilic fraction, Palestine, wild plants, α -amylase

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Introduction

Diabetes mellitus (DM) represents one of the most common non-communicable chronic diseases globally and has two main types; type I, and type II which represents the majority of all DM cases with 85%. The latest epidemiological surveys and studies on DM II indicate that the prevalence of this disease is increasing and expected to reach 592 million in 2035 [1, 2]. Long-term hyperglycemic status leads to microvascular complications such as retinopathy, nephropathy, and neuropathy, in addition to macro-vascular complications including cardiovascular events and stroke [3]. Its increased morbidity and mortality rates all over the world decrease the quality of human being's lives and productivity even in the developed countries. As a result, it is considered one of the challenging diseases in the pharmaceutical world [4, 5]. Currently, available therapeutic agents for DM include insulin and various oral antidiabetic drugs such as glinides, sulfonylureas, and biguanides; most of them have a number of harmful side effects; for this reason, the investigations of safer and effective hypoglycemic medications is one of the essential areas of research [6].

The α -amylase enzyme is produced from the human saliva and the pancreatic juice which plays an essential role in the hydrolysis of starch and glycogen into simple sugars (glucose and maltose). This process leads to increasing the sugar level in the blood, which stimulates the production of insulin from the pancreas to balance the blood sugar level by activating the sugar entry into the body cells [7].

Herbal medicine is considered one of the most important and popular branches of complementary and alternative medicine and folkloric medicine that have been established thousands of years ago [8, 9].

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The discovery of numerous pollens in graves located in the Zagros Mountains of Kurdistan in Iraq for more than 60,000 years ago indicates an awareness of herbal medicine by Neanderthals [10, 11]. The use of plants as natural anti-diabetic agents in medicine and in processed foods has become increasingly important in the pharmaceutical and food industries [12].

However, the α -amylase inhibitory activity of many plants which are utilized rationally as antidiabetic agents has been studied, and their mechanisms of actions are being confirmed [13, 14].

Recently, a number of edible and medicinal plants have been proposed to be useful in diabetes worldwide, and have been used empirically in the treatment of diabetes. Traditional medicinal and edible plants from available wild plants offer a great potential for the investigation of new anti-diabetic drugs [13, 15].

The aim of this study was to evaluate the inhibitory activity of α -amylase in the hydrophilic and lipophilic fractions of seven traditional edible and medicinal wild plants from Palestine. The plants in the study included: *A. palaestinum* Boiss, *M. sylvestris* L., *P. major* L., *C. iberica* Trevir. ex Spreng., *C. endivia* L., *B. bituminosa* (L.) C.H.Stirt, and *Sisymbrium irio* L. The current evaluation of α -amylase inhibitory activity was determined in comparison with a reference compound Acarbose which is used as a blocker of the pancreatic α -amylase enzyme [16].

To the best of authors knowledge, no previous investigations were documented about the pancreatic α -amylase enzyme inhibitory effects in the hydrophilic and lipophilic fractions of *A. palaestinum*, *M. sylvestris*, *P. major*, *C. iberica*, *C. endivia*, *B. bituminosa*, and *S. irio*. Furthermore, this study is the first to evaluate the inhibitory activity of α -amylase in these wild edible and medicinal plants.

Results

The *in vitro* anti-diabetic activity of the seven folkloric edible and rational medicinal Palestinian plants was investigated by the assessment of their α -amylase inhibitory effects. Acarbose was used as a standard drug to compare their inhibitory effects.

However, all the studied α -amylase inhibitory assays in the hydrophilic and lipophilic fractions of the seven plant species including Acarbose were conducted at a concentration of 100 $\mu\text{g/mL}$.

An evaluation of the plot of % α -amylase inhibition activities in the hydrophilic and lipophilic fractions of *A. palaestinum*, *M. sylvestris*, *P. major*, *C. iberica*, *C. endivia*, *B. bituminosa*, and *S. irio* plants as a function of each fraction concentrations are shown in Table 1 and Figure 1–Figure 2, from which the IC_{50} values were calculated (Table 2).

Table 1: α -amylase inhibitory effects of the studied plant's species.

Conc.	A	A.p		B.b		C.i		C.e	
		H.F	L.F	H.F	L.F	H.F	L.F	H.F	L.F
10	53.22 ± 0.55	14.73 ± 0.6	49.75 ± 1.48	20.49 ± 0.57	13.5 ± 0.55	63.9 ± 0.56	23.54 ± 0.9	56.73 ± 0.32	33.15 ± 0.63
50	54.91 ± 1.4	14.93 ± 0.32	49.75 ± 1.48	28.45 ± 2.61	13.5 ± 0.64	65.94 ± 0.62	28.85 ± 0.91	57.57 ± 0.86	34.2 ± 0.27
100	66.1 ± 0.64	53.64 ± 0.62	69.26 ± 2.89	52.42 ± 0.53	25.2 ± 1.44	65.94 ± 0.62	29.05 ± 0.63	58.39 ± 0.86	51.83 ± 1.44
500	72.54 ± 0.75	56.7 ± 0.28	90.75 ± 1.48	52.42 ± 0.53	37.48 ± 1.43	81.1 ± 0.56	29.05 ± 0.63	65.57 ± 2.31	57.37 ± 2.31
700	78.98 ± 2.3	89.75 ± 2.89	94.05 ± 3.18	64.75 ± 0.57	69.26 ± 2.89	82.1 ± 0.84	29.05 ± 0.63	87.7 ± 5.79	67.21 ± 5.79

M.s		P.m		S. irio	
H.F	L.F	H.F	L.F	H.F	L.F
33.79 ± 0.85	15.36 ± 0.86	17.62 ± 0.57	39.5 ± 1.41	29.9 ± 0.007	49.99 ± 0.57
52.86 ± 1.73	15.98 ± 2.89	17.62 ± 0.57	57.3 ± 0.56	38.11 ± 0.57	65.7 ± 0.86
93.23 ± 0.86	21.51 ± 2.02	36.45 ± 2.89	79.5 ± 5.7	48.36 ± 0.57	65.7 ± 0.86
93.23 ± 0.86	22.54 ± 0.57	49.95 ± 0.63	77.49 ± 2.89	82.37 ± 0.58	94.26 ± 0.58
93.64 ± 1.44	32.37 ± 3.69	90.75 ± 1.48	92 ± 0.28	90.57 ± 1.15	96.51 ± 0.28

(H.F: Hydrophilic fraction, L.F: Lipophilic fraction, A: Acarbose, A.p: *A. palestinum*, B.b: *B. bituminosa*, C.i: *C. iberica*, C.e: *C. endivia*, M.s: *M. sylvestris*, P.m: *P. major* and S.i: *S. irio*).

Figure 1: Porcine pancreatic α -amylase inhibitory effects of Acarbose, hydrophilic, and lipophilic fractions of *S. irio* leaves.

Figure 2: Porcine pancreatic α -amylase inhibitory effects of Acarbose, hydrophilic, and lipophilic fractions of *C. endivia* leaves.

The results showed that all hydrophilic plants fractions exhibited mild to excellent α -amylase inhibitory activity, with IC_{50} values ranging from 9.96 to 573.72 $\mu\text{g/mL}$. The lipophilic fractions exhibited superior potentials of *S. irio*, *A. palaestinum*, and *P. major* plants with IC_{50} values, 7.72, 25.34, and 61.35 $\mu\text{g/mL}$, respectively, as well as the IC_{50} value of Acarbose was found to be 10 $\mu\text{g/mL}$ as shown in Table 2.

Table 2: The IC_{50} values of α -amylase inhibitory results and their inhibitory concentration of the hydrophilic and lipophilic fractions of the studied plant's species.

Studied samples	Hydrophilic fraction, IC_{50} value, ($\mu\text{g/mL}$)	Lipophilic fraction, IC_{50} value, ($\mu\text{g/mL}$)
<i>P. major</i>	352.31	61.35
<i>M. sylvestris</i>	38.55	Not calculated
<i>A. palaestinum</i>	573.72	25.34
<i>S. irio</i>	180.43	7.72
<i>C. endivia</i>	9.96	300.92
<i>B. bituminosa</i>	189.89	529.49
<i>C. iberica</i>	12.33	Not calculated
Acarbose (positive control)	10.00	10.00

Discussion

Diabetes mellitus has triggered a major burden to the health-care sectors in the developing and developed countries and has shown an increasing trend among the urban population. Diabetes has reached epidemic proportions and is becoming a public health concern of the highest order. Several methods are now available to treat diabetes. This metabolic disorder reached epidemic proportions and is becoming a public health concern of the highest order. It is estimated that most patients with type II diabetes could be easily treated with extensive exercises, medications, and dietary changes [17]. Therapeutic agents that can reduce the post-prandial hyperglycemia by the suppression of starch hydrolysis such as pancreatic α -amylase inhibitors have been found to be a useful tool in controlling diabetes mellitus type II [18].

Acarbose is a fermentation by-product of *Actinoplanes* species which is a commercially available α -amylase inhibitor that works by reducing the absorption of carbohydrate in small intestine by inhibiting both α -glucosidase enzyme and pancreatic α -amylase enzyme; which is considered very important in the treatment of type II diabetes mellitus by controlling postprandial blood glucose level by preventing the absorption of amylum carbohydrate [16]. However, it is reported to cause various side effects such as abdominal distention, flatulence, and possibly diarrhea. Hence, searches for safe and effective inhibitors from natural sources are of emerging interest [19].

In fact, plants have played an important role in the discovery and development of a large number of medications. They have always been a common source of drugs, either in the form of traditional remedies or as pure active constituents [20–22].

The consumption of plants or herbal supplements may be a more acceptable source of α -amylase inhibitors due to their low cost; furthermore, all the studied plant species were used from ancient times as edible and medicinal folkloric herbals in Palestine [23].

In fact, the lipophilic fraction of *S. irio* has a very powerful porcine pancreatic α -amylase inhibitory effect with IC_{50} value of 7.72 $\mu\text{g/mL}$ in comparison with Acarbose, while the hydrophilic fraction of this plant has too much less α -amylase inhibitory effect with IC_{50} value of 180.43 $\mu\text{g/mL}$.

In the Middle Eastern countries, *S. irio* is utilized as folkloric food and for treatment of rheumatism, cold symptoms, diabetes, and for the detoxification of the liver and spleen. It is an annual herbaceous plant belonging to Brassicaceae family which has small pale yellow flowers and broad leaves at the base of stalk while linear in shape at the upper part of stalk [24].

However, in modern medicine, it has proved that it has antipyretic, analgesic, antibacterial action against both Gram-positive and negative microorganisms, antioxidant activity due to the high amount of phenols, flavonoid, and sulpharophanes [25–27].

Moreover, the hydrophilic fraction of *M. sylvestris* has powerful porcine pancreatic α -amylase inhibitory effect with an IC_{50} value of 38.55 $\mu\text{g/mL}$ in comparison with Acarbose (10 $\mu\text{g/mL}$), while the lipophilic fraction of this current plant was inactive.

However, *Malva sylvestris* (family Malvaceae) is an annual herbaceous plant widely distributed in Palestine. Its leaves are used as a folkloric food and medicine from the ancient times due to its curable and nutritional values. It used for the treatment of irritable bowel syndrome, cough, irritable throat, and as a hypoglycemic agent. The leaves contain 2-methoxy-4-vinylphenol, gossypin-3-sulphate, hypolaetin-8-O-b-D-glucoside-3'-sulphate, gossypetin-8-O-b-D-glucouronide-3-sulphate, and rich in mucilage [28–30].

The *P. major* plant belongs to plantaginaceae family which is a popular traditional edible and medicinal plant. The leaves of decoction are traditionally used for the treatment of various dermatological diseases, upper respiratory system infections, digestive problems, cancer, diabetes mellitus, and pain [31].

In fact, many conducted studies on the effects of *P. major* leaves have been established to prove its therapeutic properties such as anti-inflammatory, antiulcerogenic, immuno-modulating, antioxidant, anticancer, anti-cough, anti urolithiasis, and *in-vivo* hypoglycemic properties [24–26]. The *P. major* leaves contain biologically active molecules including flavonoids, tannins, triterpenic acids, iridoid glycosides, and complex polysaccharide (mucilage) [31–35].

However, the lipophilic fraction of *P. major* revealed potential α -amylase inhibitory effect with an IC_{50} value of 61.35 $\mu\text{g/mL}$ which is six times weaker than the positive control Acarbose.

The leaves' methanolic extract showed strong antibacterial activity against *Erwinia carotovora*, *Entero. faecalis*, *Staph. Aureus*, and *Strep. agalactiae*. In addition, it showed antifungal efficacy against *Aspergillus candidus*, *Aspergillus niger*, *Penicillium spp.*, and *Fusarium culmorum* colonies. Also, it revealed potent antioxidant and anti-inflammatory effects [36, 37].

In addition, the hydrophilic fraction of *A. palaestinum* has powerful porcine pancreatic α -amylase inhibitory effect with an IC_{50} value of 573.72 $\mu\text{g/mL}$ in comparison with Acarbose (10 $\mu\text{g/mL}$), while the lipophilic fraction of this plant was near the value of α -amylase inhibitory effect with an IC_{50} value of 25.34 $\mu\text{g/mL}$.

Arum palaestinum is one of the most popular edible wild plants in Palestine which is also used for the treatment of various diseases including diabetes, cancer, obesity, and gastrointestinal inflammations [38]. It belongs to Araceae family and grows about 25 cm height. It blooms in the spring months of March and April [39]. Phytochemical analysis of *A. palaestinum* leaves by liquid chromatography-tandem mass spectrometry (UH-PLC–DAD-MS/MS) identify the presence of flavonoids, phenolic acids, terpenoids, iridoids, amino acids, and alkaloids, which are considered a rich source of biologically active compounds [26, 40].

However, the methanol extract of *A. palaestinum* leaves has a moderate level of antioxidant which has an important role in scavenging free radicals such as the reactive oxygen species which is responsible for many tissue damages. Also, there is a linear relationship between the phenolic component and antioxidant activity of the methanol extract, which gives the plant anti-diabetic role [41].

The porcine pancreatic α -amylase inhibitory effect IC_{50} value $9.96 \mu\text{g/mL}$ of the hydrophilic fraction of *C. endivia* leaves is almost stronger than Acarbose.

Cichorium endivia is an annual or biennial herbaceous plant; it belongs to Compositae family. Its leaves are valued as a green salad in Palestine. It is an erect herb up to 1.7 m tall, with a rosette of large leaves and taproot, containing a bitter milky juice. It produces attractive pale blue flowers on stems that stand way above the leafy foliage [42]. The leaves are widely used in the traditional medicine to treat skin infections and diabetes [43].

Moreover, due to its high contents of the antioxidant compound, it has anticancer, hepatoprotective, and anti-inflammatory properties [44].

In addition, it has high contents of flavonoid glycosides including quercetin, kaempferol 3-O-[(6-O-malonyl)glucoside], quercetin 3-O-glucoside, kaempferol, quercetin 3-O-glucuronide, cyanidin 3-O-[(6-O-malonyl)glucoside], quercetin 3-O-(6-O-malonyl)glucoside, quercetin 3-O-rhamnoside, luteolin 7-O-glucuronide, kaempferol 3-O-glucoside, kaempferol 3-O-glucuronide, and quercetin 3-O-galactoside [45].

In fact, the hydrophilic fraction of *B. bituminosa* has mild activity against porcine pancreatic α -amylase with an IC_{50} value of $189.89 \mu\text{g/mL}$ while the lipophilic fraction was too high.

Furthermore, the hydrophilic fraction of *C. iberica* has a very powerful and the best porcine pancreatic α -amylase inhibitory effect with an IC_{50} value of $12.33 \mu\text{g/mL}$ in comparison with Acarbose, while the lipophilic fraction of this plant was inactive.

Centaurea iberica belongs to Compositae family which is a biennial small shrub widely distributed in the waste places and around the cultural field in the West Bank area of Palestine. It is used extensively as an alternative medicine for treatment of abdominal pains, insect or snake bites, and wounds in Palestine and Turkey [43, 46], while known to be stomachic, antidiabetic, and choleric agent in Lebanon [47]. The edible parts of the plant are the stems and leaves. The leaves have hispidulous to loosely tomentose shape and slightly bitter taste [46].

Evidence-based medicine has shown that its extract has anti-inflammatory and wound-healing properties [48], in addition to its platelet aggregation inhibitory effect. The plant contains 3-methyl-2-benzyl-4-quinazolinone and methyl-2-[(methylamino) carbonyl] benzoate, along with a dimeric lignan glucoside [49].

Furthermore, *A. palaestinum*, *M. sylvestris*, *P. major*, *C. iberica*, *C. endivia*, *B. bituminosa*, and *S. irio* leaves contain biologically active molecules, especially flavonoids and phenols, and some of them contain heteroglycan complex polysaccharide, especially mucilage.

Eventually, the ingestion of heteroglycan complex polysaccharide such as mucilage found that they can delay the diffusion of glucose, inhibit the activities of α -amylase, pancreatic lipase and protease, sequester bile acids, and reduce the amount of cholesterol available for absorption [50].

In addition, many previously conducted investigations demonstrated a direct relationship between antioxidant molecules, polyphenols and flavonoids contents, and porcine pancreatic α -amylase inhibitory activity [51, 52].

In a study conducted by Tadera et al., it was found that the presence of hydroxyl group and an unsaturated gamma-pyrone ring of the main chemical nucleus of flavonoids enhanced their porcine pancreatic α -amylase inhibitory activity [53].

Additionally, many previously established studies suggested that the polyphenolic molecules with the highest number of hydroxyl groups gave the greatest porcine pancreatic α -amylase inhibitory effect [54].

Moreover, functional foods could ultimately be developed containing components able to inhibit α -amylase, an Acarbose-like action but without the side effects.

Currently, the improvement of glucose homeostasis by decreasing the intestinal absorption of dietary glucose with alternatives to Acarbose through the inhibition of porcine pancreatic α -amylase is of increasing interest. Further clinical trial studies are required to approve the current studied plant species pharmacological anti-diabetic action and also to assess their safe consumption.

Conclusion

Several plants are used in folk medicine to treat diabetes and the use of edible plants is of great importance since they have fewer adverse effects than those that may be encountered with chemical or drug treatments. Our obtained results show that some edible plants could be used in the treatment of diabetes after clinical trials. Prospective fractions of hydrophilic and lipophilic herbs were analyzed by *in-vitro* enzyme assay and according to the obtained results, *A. palaestinum*, *M. sylvestris*, *P. major*, *C. iberica*, *C. endivia*, *B. bituminosa*, and *S. irio* may be used as natural inhibitors of pancreatic lipase and so are new treatments in DM. Accordingly, due to their inhibitory effects on the α -amylase level, they could be used as a monotherapy along with an appropriate

diabetic diet and exercise or might be used in conjunction with anti-diabetic therapy to manage and prevent progression of diabetes.

Material and methods

Plants collecting and preparing

The leaves of *A. palaestinum*, *M. sylvestris*, *P. major*, *C. iberica*, *C. endivia*, *B. bituminosa*, and *S. irio* plants were collected in May 2017 from different regions of Palestine. Taxonomical identifications were established by the pharmacognosist Dr Nidal Jaradat at the Pharmacognosy Laboratory at An-Najah National University and the voucher specimen codes assigned were: Pharm-PCT-246, Pharm-PCT-1507, Pharm-PCT-1888, Pharm-PCT-548, Pharm-PCT-617, Pharm-PCT-394, and Pharm-PCT-2288, respectively.

The plant's leaves were washed well and then dried in the shade at controlled temperature ($25 \pm 2^\circ\text{C}$) and humidity ($55 \pm 5\text{ RH}$). After drying process, the leaves were grounded well by using a mechanical grinder into a fine powder and then transferred into airtight containers with proper labeling for future use.

Chemicals

All chemicals were bought from different commercial sources: Ethanol (Loba Chemie, Mombay, India), n-hexane (Frutarom LTD, Haifa, Israel), DMSO (Riedel-de-haen, Seelze, Germany), α -amylase (Sigma, Mumbai, India), DNSA (3,5-dinitrosalicylic acid) reagent (Sigma, LA, USA), and Acarbose (Sigma, St. Louis, USA).

Equipment

Different equipments were used; all of these equipments are present in An-Najah National University's laboratories like Shaker device (Memmert shaking incubator, Buchenbach, Germany), UV-visible spectrophotometer (Jenway 7135, Staffordshire, UK), grinder (Moulinex, model LM2211, UNO, Shanghai, China), balance (Radwag, AS 220/c/2, Radom, Poland), freeze-dryer (Mill rock technology, model BT85, Danfoss, Shanghai, China), filter paper (Machrery-Nagel, PA, USA; MN 617), and rotary evaporator (Heidolph OB2000, VV2000, Schwabach, Germany).

Fractionation procedure

A total of 25 g of the powdered plant was weighed and then fractionated by adding 100 mL of n-hexane and 150 mL of 50% ethanol in triple distilled water. The mixture was then shaken for 48 h at room temperature using a shaker that was set at 200 rpm. Subsequently, the mixture was filtered using a suction flask and Buchner funnel filtration. The obtained filtrate was separated individually by separation funnel into two phases; a lower hydrophilic phase representing the first aqueous fraction and an upper lipophilic phase representing the organic fraction. The remaining solid materials were re-fractionated separately by 150 mL of 50% ethanol in triple distilled water and this re-fractionated process was carried out as described above. The hydrophilic fraction was lyophilized into dry powder, while the lipophilic fraction was kept in the hood at 25°C to evaporate leftover organic solvents [55].

The lipophilic fraction's yields of *A. palaestinum*, *M. sylvestris*, *P. major*, *C. iberica*, *C. endivia*, *B. bituminosa*, and *S. irio* were 18.77%, 7.25%, 21.6%, 9.45%, 14.25%, 16.66%, and 16.9%, respectively, while the hydrophilic fraction's yields for the same plants were 9.44%, 15.12%, 6.33%, 13.7%, 12.6%, 7.5%, and 6.11%, respectively.

In vitro α -amylase inhibitory effect in the lipophilic and hydrophilic plants fractions

The α -amylase inhibition assay was performed using the 3,5-dinitrosalicylic acid (DNSA) method. For each plant, both lipophilic and hydrophilic fractions were dissolved in minimum amount of 10% DMSO and then further dissolved in buffer ($(\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ (0.02 M), NaCl (0.006 M) at pH 6.9) to give concentrations of 1,000 $\mu\text{g/mL}$ from which the following dilutions were prepared (10, 50, 100, 500, 700 $\mu\text{g/mL}$). A volume of

200 μL of porcine pancreatic α -amylase enzyme solution (2 units/mL) was mixed with 200 μL of the plant fraction and was incubated for 10 min at 30 °C. Thereafter, 200 μL of the freshly prepared starch solution (1% in water (w/v)) was added to each tube and incubated for 3 min. The reaction was terminated by the addition of 200 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) and was then diluted with 5 mL of distilled water and subsequently boiled for 10 min in a water bath at 85–90 °C. The mixture was cooled to ambient temperature, and the absorbance was measured at 540 nm using a UV-Visible spectrophotometer. The blank with 100% enzyme activity was prepared by replacing the plant fraction with 200 μL of buffer. A blank reaction was similarly prepared using the plant fraction at each concentration in the absence of the enzyme solution [49]. A positive control sample was prepared using Acarbose following the same previous steps and the reaction was performed similarly to the reaction with plant fractions as mentioned above.

The α -amylase inhibitory activity was expressed as percent inhibition and was calculated using the equation given below:

$$\% \text{ of } \alpha\text{-amylase inhibition} = (B - S) / B * 100\%$$

where

B: is the absorbance of blank and S: is the absorbance of the tested sample.

Statistical analysis

The obtained results of the studied plant's lipophilic and hydrophilic fractions were expressed as means $\pm$ standard deviation (SD). Averaged data were compared using t-test. The statistical significance was considered when the p-value was <0.05 .

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