Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.apjtb.com

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In vitro anti-diabetic, anti-obesity and antioxidant proprieties of *Juniperus phoenicea* L. leaves from Tunisia

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ARTICLE INFO

Article history:
Received 18 Mar 2014
Received in revised form 5 May 2014
Accepted 29 May 2014
Available online 28 Jun 2014

Keywords:
Chemical composition
α-Amylase
Lipase
Essential oil
Antioxidant activity

ABSTRACT

Objective: To examine chemical composition and antioxidant activity as well as the *in vitro* α-amylase and pancreatic lipase inhibitory activities of the essential oil and various extracts of *Juniperus phoenicea* (*J. phoenicea*).
Methods: Essential oil obtained by steam distillation were analyzed by gas chromatography-mass spectrometry technique. The antioxidant activity of the essential oil and various extracts of *J. phoenicea* were determined by DPPH and β-carotene bleaching methods.
Results: Gas chromatography-mass spectrometry analysis of the *J. phoenicea* essential oil resulted in the identification of 37 compounds, representing 96.98% of the oil; α-Pinene (24.02%), limonene (7.94%), D-3-Carene (16.9%), Germacrene D (11.98%), Germacrene B (5.40%) and δ-cadinene (6.52%) were the major compounds. The IC_{50} values of essential oil, hexane and methanol extracts against α-amylase were 35.44, 30.15 and 53.76 μg/mL respectively, and those against pancreatic lipase were 66.15, 68.47 and 60.22 μg/mL respectively, suggesting powerful anti-diabetic and anti-obesity effects. Antioxidant activity (IC_{50}=2 μg/mL) and total phenolics content (265 mg as gallic acid equivalent/g extract) of the methanol extract were found to be the highest compared to the other extracts.
Conclusions: The findings showed that the extents of α-amylase and pancreatic lipase inhibitory activities of the *J. phoenicea* extracts as well as their antioxidant activity are in accordance with total phenolics contents. Leaves of *J. phoenicea* being rich in phenolics may provide a good source of natural products with interesting medicinal properties.

1. Introduction

Diabetes mellitus is a serious health problem that has adverse and long-lasting consequences for individuals, families, and communities. The magnitude of this problem has increased dramatically over the last three decades and estimated to reach 439 million among adult patients by 2030[1]. This health disease is a chronic metabolic disorder caused by the total (or relative) defect of insulin, which is manifested clinically as elevated blood glucose[2]. The diabetic patients exhibited a higher risk in the development of several chronic health complications including obesity, atherosclerosis, dyslipidemia and renal failure worldwide[3].

Inhibition of enzymes involved in the metabolism of carbohydrates such as α-amylase is an important therapeutic approach for reducing postprandial hyperglycemia[2]. Moreover, one of strategies used in the discovery of anti-obesity drugs is to search for potent lipase inhibitors from plant extracts. In fact, several synthetic drugs such as acarbose and orlistat are widely used as inhibitors of these enzymes in patients with Type 2 diabetes and obesity[4,5]. However, these inhibitors are reported to cause several side effects[6]. Therefore, many efforts has been focused to minimize side effects of these hypoglycemic and anti-obesity synthetic drugs and to find natural and safer α-amylase and lipase inhibitors. A largest number of medicinal plants are used in managing

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Foundation Project: Supported by the Ministry of High Education and Scientific Research, Tunisia (Grant NO. UR11-ES74).

diabetes mellitus and its related complications, due to their photochemical active contents such as phenolics and flavonoids with strong antioxidant properties[7]. These compounds have been reported to be effective inhibitors of α-amylase and lipase[8].

Juniperus phoenicea (*J. phoenicea*) is considered as an important medicinal plant largely used in the Tunisian traditional medicine. Its leaves are used in the form of decoction to cure many diseases such as diarrhea, bronchitis, rheumatism[9,10], and diabetes[11,12]. Hence, this study sought to investigate the *in vitro* inhibitory ability of various extracts of *J. phonicea* on α-amylase and lipase activities as well as to assess their antioxidant property.

2. Materials and methods

2.1. Collection of plant material

Leaves of *J. phoenicea* L. (Cupressaceae) were used in this study. Plant was collected in October, 2011, from local *Ain Silisla El Oyoun* Company in government of Kasserine in mid-west Tunisia. Voucher specimen was deposited at the Herbarium of the Life Sciences department in the Faculty of Sciences of Sfax.

2.2. Chemicals

2-Chloro-4-Nitrophenyl-α-D-Maltotrioside (CNPG3) was purchased from Biolabo reagents France, Acarbose from Pharmaghreb Tunisia, dimethylsulfoxide (DMSO) from Merck (Germany) and porcine pancreatic α-amylase (PPA) and porcine pancreatic lipase was procured from Sigma-Aldrich, St. Louis, USA. All the other chemicals used were of analytical grade.

2.3. Extraction

The dried leaves powder of *J. phoenicea* (130 g) was extracted sequentially by maceration in hexane (4×600 mL), ethyl acetate (EtOAc) (4×600 mL) and MeOH (4×600 mL) at room temperature and concentrated under vacuum at 40 °C to afford 6.25, 6.88 and 21.8 g, respectively.

2.4. Extraction of essential oil

The fresh *J. phoenicea* leaves were completely immersed in water and hydro-distilled for 4 h in a Clevenger-type apparatus giving yellow oil. When the condensed material cooled down, the water and essential oil were separated. The oil was decanted to be used as essential oil. To improve its recovery, the essential oil was taken up in diethyl ether, dried over anhydrous sodium sulphate until the last traces of water were removed and stored in a dark glass bottle at 4 °C until tested and analyzed.

2.5. Gas chromatography-mass spectrometry (GC-MS)

Analysis of the *J. phoenicea* essential oil was performed on a GC-MS HP model 5975B inert MSD (Agilent Technologies, J&W Scientific Products, Palo Alto, CA, USA), equipped with an Agilent Technologies capillary DB-5MS column (30 m length; 0.25 mm inner diameter; 0.25 mm film thickness), and coupled to a mass selective detector (MSD5975B, ionization voltage 70 eV; all Agilent, Santa Clara, CA). The carrier gas was He and was used at 1 mL/min flow rate. The oven temperature program was as follows: 1 min at 100 °C ramped from 100 to 260 °C at 4 °C/min and 10 min at 260 °C. The chromatograph was equipped with a split/splitless injector used in the split mode. The split ratio was 1:100. Identification of components was assigned by matching their mass spectra with Wiley and NIST library data, standards of the main components and comparing their Kovats Retention Indices (KRI) with reference libraries[13,14], and from the literature. The components concentrations were obtained by semi-quantification by peak area integration from GC peaks and by applying the correction factors.

2.6. α-Amylase inhibition assay by CNPG3 method

The *in vitro* α-amylase inhibition activity of all extracts was determined based on the spectrophotometric assay using acarbose as the reference compound[15]. The plant extract was dissolved in DMSO to give concentrations from 50, 100 and 200 μg/mL. The enzyme α-amylase solution was prepared by mixing of α-amylase in 100 mL of 40 mmol/L phosphate buffer, pH 6.9. Positive control, acarbose was obtained by dissolving in phosphate buffer. The assays were conducted by mixing 80 μL of plant extract, 20 μL of α-amylase solution and 1 mL of CNPG3. The mixture was incubated at 37 °C for 5 min. The absorbance was measured at 405 nm spectrophotometrically (Jenway 6405 UV/Visible, Great Britain). Similarly, a control reaction was carried out without the plant extract/acarbose. Percentage inhibition (PI) was calculated by the expression:

$$PI=[(Absorbance_{Control}-Absorbance_{Test})/Absorbance_{Control}]\times100$$

2.7. Pancreatic lipase in vitro assay

The method was modified from the assay reported by Nakai *et al*[16], in which 4-methylumbelliferyl oleate (4-MU oleate) was used as a substrate to measure the pancreatic

lipase inhibitory activity of all the extracts. Briefly, the assay was conducted by mixing 50 μL of the pancreatic lipase solution (2 IU/mL) in a buffer consisting of 50 mmoL/L Tris HCl (pH 8.0), 100 μL of diluted sample solutions and 50 μL of 0.5 mmoL/L 4-MU solution dissolved in the above buffer in the well of a 96 micro well plate to start the enzyme reaction. The plate was immediately placed in the 37 °C pre-heating FL 800× micro plate fluorescence reader (Bio-Tek® Instruments, Inc., Winooski, VT) to measure the amount of 4-MU released by lipase every minute for 30 min at an excitation wavelength of 360 nm with a tolerance of ±40 nm and an emission wavelength of 455 nm with a tolerance of ±20 nm. The lipase inhibitive activity was determined by measuring the effect on the enzyme reaction rate after adding extracts, compared with the control. Orlistat was used as positive control. PI was calculated by the expression:

$$PI=[(Absorbance_{Control}-Absorbance_{Test})/Absorbance_{Control}]\times100$$

2.8. Determination of total phenolics

Total phenolics contents were assayed using the Folin-Ciocalteu reagent and gallic acid as a standard following Singleton's method slightly modified by Oktay *et al*[17]. Folin-Ciocalteu reagent (0.5 mL) was added to a solution containing 1 mL of extract, with a known concentration (1 mg/mL) and 3 mL of distilled water. The solution was mixed and after 3 min, 0.5 mL of 2% sodium carbonate solution was added. The mixture was left to incubate for 90 min, and the absorbance was measured at 760 nm. The total phenolics content was calculated by a standard gallic acid graph, and the results expressed in mg of gallic acid equivalents per g (mg GAE/g) of dry weight of extract. The assay was performed in triplicate for each extract.

2.9. Determination of total flavonoids

The total flavonoids contents in the various extracts were determined according to Akrout *et al*[18], using a method based on the formation of a complex flavonoid-aluminium, having the maximum absorbance at 430 nm. Quercetine was used to make the calibration curve. About 1 mL of diluted sample was mixed with 1 mL of 2% aluminium trichloride ($AlCl_3$) methanol solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm with a Shimadzu UVmin-1240 UV-Vis spectrophotometer and the total flavonoid content was expressed in mg quercetine equivalent (QE) per g of extract.

2.10. Antioxidant activity determination

2.10.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

DPPH radical-scavenging effect was evaluated following the procedure described in a previous study[19]. Fifty microliters of various concentrations of the essential oil and the extracts dissolved in methanol were added to 5 mL of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical, DPPH, in percent (I%) was calculated in the following way:

$$I\%=(A_{blank}-A_{sample})/A_{blank}\times100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test extract), and A_{sample} is the absorbance of the test extract. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted of inhibition percentage against extract concentration. The synthetic antioxidant reagents butylate hydroxytoluene (BHT) and vitamine E were used as positive control and all tests were carried out in triplicate.

2.10.2. β-Carotene bleaching method

The antioxidant activity of *J. phoenicea* essential oil and various extracts was evaluated by the β-carotene method as described by Allouche *et al*[20]. β-Carotene (0.2 mg) in 0.2 mL of chloroform, linoleic acid (20 mg), and Tween-40 (polyoxyethylene sorbitan monopalmitate) (200 mg) were mixed. Chloroform was removed at 40 °C under reduced pressure (40 mmHg), and the resulting mixture was diluted with 10 mL of water and mixed well. This emulsion was added with 40 mL of oxygenated water. Aliquots (4 mL) of the emulsion were transferred into different test tubes containing 0.2 mL solutions of *J. phoenicea* extracts and BHT. A control containing 0.2 mL of ethanol and 4 mL of the above emulsion was prepared. The tubes were placed in a water bath maintained at 150 °C, and the absorbance was measured at 470 nm at zero time (t=0). Measurement of absorbance was continued until the color of the β-carotene disappeared in the control tubes (t=180 min) at intervals of 15 min. A mixture prepared as above without β-carotene served as the blank. All determinations were carried out in triplicate. The antioxidant activity (AA%) of the extracts was evaluated in terms of bleaching of the β-carotene using the following formula:

$(AA\%)=[1-(A^0-A^t)/(A_0^0-A_t^0)]$, where A^0 and A_0^0 are the absorbance values measured at zero time of the incubation for test sample and control, respectively, and A^t and A_t^0 are the absorbance values measured in the test sample and control, respectively, after incubation for 180 min.

2.11. Statistical analyses

All analyses were performed in triplicate and data were reported as means±SD. Differences between experiments were analyzed using Student's *t*-test in Microsoft Excel 2000 (Microsoft Corporation, USA). The confidence limits used in this study were based on 95% ($P<0.05$).

3. Results

The yield of *J. phoenicea* leaf essential oil was 1 mL/100 g fresh weight. The oil was a pale yellow color. Table 1 shows the constituents of the essential oil and their percentage composition as well as their Kovats Index (KI) values listed in order of elution. A total of 37 compounds representing 96.98% of the essential oil were identified. The major compounds were: α-Pinene (24.02%), limonene (7.94%), D-3-Carene (16.90%), Germacrene-D (11.98%), Germacrene-B (5.40%) and δ-cadinene (6.52%).

Table 1

Chemical composition of *J. phoenicea* L. essential oil.

No	KI	Components	Compositions (%)
1	934	α-Pinene	24.02
2	954	Camphene	1.84
3	1005	D-3-Carene	16.90
4	1029	Limonene	7.94
5	1039	E-β-Ocimene	0.03
6	1042	Linalyl acetate	0.51
7	1047	γ-Terpinene	0.15
8	1078	α-Terpinolene	3.07
9	1096	cis-Rose oxide	2.63
10	1098	Fenchol	0.30
11	1151	Pinocarvone	0.20
12	1170	Terpinen-4-ol	0.25
13	1177	Myrtenol	0.15
14	1185	Verbenone	0.20
15	1208	β-Citronellol	1.83
16	1299	Carvacrol	0.27
17	1299	E-Anethole	0.04
18	1376	α-Yalangene	0.29
19	1377	α-Copaene	0.88
20	1388	β-Bourbonene	0.29
21	1388	β-Cubebene	0.30
22	1410	β-Gurjunene	1.94
23	1418	β-Elemene	0.80
24	1418	β-Cedrene	0.32
25	1419	Caryophyllene	3.75
26	1422	γ-Elemene	0.55
27	1441	Aromadendrene	0.46
28	1442	α-Humulene	2.85
29	1470	α-Amorphene	0.83
30	1480	α-Muurolene	1.66
31	1485	Germacrene-D	11.98
32	1493	Cadina-1,4-diene	0.80
33	1523	δ-Cadinene	0.88
34	1530	Elemol	1.02
35	1551	γ-Cadinene	0.87
36	1554	Germacrene-B	5.40
37	1635	τ-Muurolol	0.84
Total	-	-	96.98

KI=Kovats Index on DB-5MS column in reference to *n*-alkanes.

Based on the absorbance values of the various extracts solutions compared with the standard solutions of gallic acid and quercetin, the total phenolics and flavonoids are shown in Table 2. Except the essential oil, the other extracts were found to be rich in flavonoids and polyphenols. The amounts of total phenolics [(265.0±5.8) mg GAE/g] and flavonoids [(176.00±0.52) mg QE/g] contents were highest in the polar methanol extract followed by the EtOAc extract [(180.8±3.6) mg GAE/g, (104.0±0.8) mg QE/g] and the hexane extract [(162.3 ±3.2) mg GAE/g, (96.00±0.48) mg QE/g].

Table 2

Total phenolics and flavonoids contents of various extracts of the leaves of *J. phoenicea*.

Extracts	Total phenolics[a]	Total flavonoids[b]
Hexane extract	162.3±3.2	96.00±0.48
Ethyl acetate extract	180.8±3.6	104.00±0.80
Methanol extract	265.0±5.8	176.00±0.52

Mean±SD were obtained from three different experiments. [a]: Total phenolics expressed as mg GAE/g extract. [b]: Total flavonoids expressed as mg QE/g extract.

Free radical scavenging activity (DPPH) of the leaves extracts and essential oil of *J. phoenicea* were tested using DPPH method and the obtained results are represented in Figure 1 as IC_{50} (μg dry weight extract/mL). The polar methanol extract exhibited the strongest free radical-scavenging activity with an IC_{50} value of 2 μg/mL, followed by the medium-polar EtOAc extract (IC_{50}=220 μg/mL) when compared to BHT (IC_{50}=17 μg/mL) and vitamin E (IC_{50}=26 μg/mL). However, the essential oil and hexane extract did not show any activity with DPPH method.

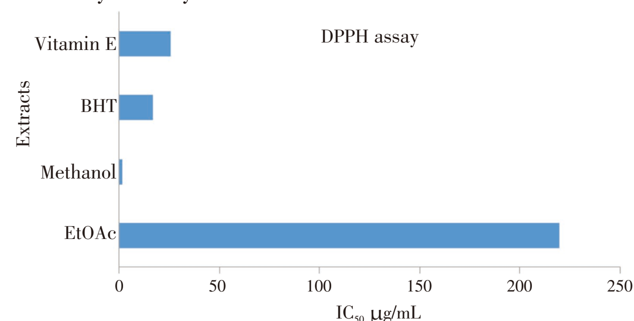

Figure 1. Free radical-scavenging capacities of the extracts measured in DPPH assay.

Values represent mean±SD for triplicate experiments about vitamin E, BHT, methanol and EtOAc extract.

The result of β-carotene bleaching method of essential oil and various extracts from *J. phoenicea* is given in Figure 2.

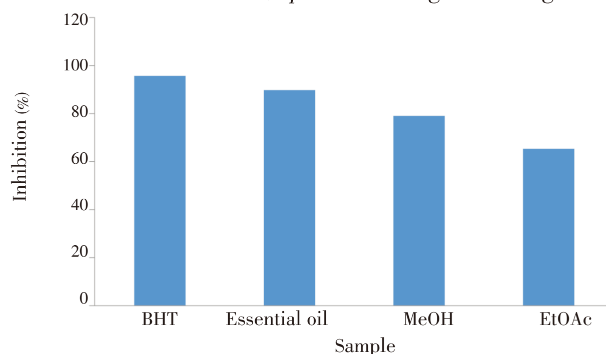

Figure 2. Inhibition ratio of the linoleic acid oxidation by the essential oil and extracts.

Values represent mean±SD for triplicate experiments about BHT, essential oil, MeOH and EtOAc extract.

The essential oil was endowed with the powerful inhibition capacity against linoleic acid (89.9%) followed by the methanol (79.2%) and EtOAc (65.3%) extracts. Hexane extract did not show any antioxidant activity.

Table 3 indicates that except EtOAc extract, all the *J. phoenicea* extracts showed a potent inhibition of α-amylase enzyme. In fact, the IC_{50} values of the *J. phoenicea* essential oil, hexane and methanol extracts against α-amylase were 35.44, 30.15 and 53.76 μg/mL respectively. However, EtOAc fraction did not exhibit any effective inhibitory action against α-amylase.

Table 3

Alpha-amylase inhibition assay of essential oil and various extracts of *J. phonicea*.

Samples	Concentration (μg/mL)	% Inhibition	IC_{50} (μg/mL)
Acarbose	25	84.00±2.10	
	50	88.72±2.07	14.88
	100	90.35±1.75	
Hexane extract	50	82.90±1.31	
	100	91.60±1.87	30.15
	200	93.70±1.13	
Methanol extract	50	46.50±1.12	
	100	93.40±1.34	53.76
	200	94.00±1.08	
Essential oil	50	46.50±1.54	
	100	93.40±1.63	35.44
	200	94.60±1.78	
EtOAc extract	-	Negligeable	-

The data are expressed in mean±SEM; *n*=3 in each group.

Table 4 shows that the IC_{50} values of hexane extract, methanol extract and essential oil were 68.47 μg/mL, 60.22 μg/mL and 66.15 μg/mL, respectively, indicating a powerful inhibition activity against lipase. EtOAc extract exhibited negligible activity.

Table 4

Pancreatic lipase inhibition assay of various extracts of *J. phonicea* and orlistat.

Samples	Concentration (μg/mL)	% Inhibition	IC_{50} (μg/mL)
Orlistat	0.1	11.53±0.74	
	1.0	58.98±0.83	0.85
	10.0	87.46±1.03	
Hexane extract	50.0	36.51±1.25	
	100.0	71.64±1.17	68.47
	200.0	86.72±1.34	
Methanol extract	50.0	41.37±1.02	
	100.0	73.40±1.27	60.22
	200.0	92.76±1.18	
Essential oil	50.0	37.79±1.03	
	100.0	85.44±1.27	66.15
	200.0	94.53±1.59	
EtOAc extract	-	Negligible	-

The data are expressed in mean±SEM; *n*=3 in each group.

4. Discussion

The chemical composition analysis showed that *J. phoenicea* essential oil contains 53.79% of monoterpene hydrocarbons. α-Pinene, and D-3-Carene were the major compounds of this fraction in the leaf essential oil. The oxygenated monoterpenes and sesquiterpenes fractions represented 6.19% and 32.25% of the total essential oil, respectively. The major sesquiterpenes were Germacrene-D and δ-cadinene. The identified compounds are known and were reported in a previous study[21]. The composition of the studied Tunisian *J. phoenicea* L plant was different from those sited in the literature[21,22]. This can be explained by the region and period of plant harvest.

The amounts of total phenolics (265 mg/g) and flavonoids (176 mg/g) contents were highest in the polar methanol extract. However, the lowest values were measured in the hexane extract. This result is in agreement with previous investigation which reported that polar solvents are among the most employed for polyphenols extraction. Moreover, it has been noted that the extraction yield of polyphenols increases with increasing polarity of the extractant solvent[7]. In a previous study, Gilles and co-workers have reported the presence of three phenylpropane glycosides (juniperoside, rosarin and skimmin) and two furanone glucoside derivatives (psydrin and phoenicein) in the *J. phoenicea* leaves[23]. Recently, oxygenated diterpenes and other phenolic compounds were isolated from Moroccan *J. phoenicea* leaves[24].

The free radical-scavenging activity of *J. phoenicea* leaves extracts was determined by the DPPH test. This test aims at measuring the capacity of the extracts to scavenge the stable radical 2.2-diphenyl-1-picrylhydrazil formed in solution by donation of an hydrogen atom or an electron[19]. If the extracts have the capacity to scavenge the DPPH free radical, the initial blue/purple solution will change to a yellow color due to the formation of diphenylpicrylhydrazine. This reaction is used as a measurement of the extracts ability to scavenge any free radical. The polar fraction related to the methanol extract containing the highest total phenolics and flavonoids contents which exhibited the strongest free radical-scavenging activity with an IC_{50} value of 2 μg/mL, followed by the medium-polar fraction representing the EtOAc extract. However, the essential oil and hexane extract, being insoluble in aqueous solution, did not show any activity with the DPPH method. Our investigation showed that the presence of phenolic compounds is fundamental for free radical-scavenging activity. All the phenolic classes have received considerable attention because of their physiological functions, including free radical scavenging. The antioxidant activity of phenolics is mainly due to their

redox properties which make them act as reducing agents, hydrogen donors, and singlet oxygen quenchers. They may also have a metal chelating potential[25].

The inhibitory effect on lipid per-oxidation of *J. phoenicea* leaves extracts was determined by the β-carotene/linoleic acid bleaching test. This test simulates the oxidation of the membrane lipid components in the presence of antioxidants inside the cells. β-Carotene, in this model system, may be oxidized and, in the absence of an antioxidant, the test solution undergoes rapid discoloration. The presence of samples with antioxidant activity can hinder the extent of β-carotene bleaching by neutralizing the linoleate-free radical formed in the system[26]. Among the tested extracts, the essential oil of *J. phoenicea* was endowed with the powerful inhibition capacity against linoleic acid (89.9%) and hexane extract did not show any antioxidant activity. The essential oil antioxidant activity was comparable to that of BHT (94.7%). This activity can be explained by the presence of hydroxylated compounds such us terpenoids (α-pinene and D-3-Carene) in the *J. phoenicea* essential oil[27]. Complementary investigations are necessary to assess the effectiveness of this oil in food system.

This assay evaluated the ability of *J. phoenicea* extracts to inhibit the activity of α-amylase, a digestive enzyme secreted from the pancreas and salivary gland. α-Amylase is involved in important biological processes such as digestion of carbohydrates. Many crude drugs inhibit α-amylase activity[28]. Natural α-amylase inhibitors are beneficial in reducing post-prandial hyperglycemia by delaying the digestion of carbohydrates and, consequently, the absorption of glucose. Hexane extract and essential oil are endowed with the powerful α-amylase inhibition activity. However, EtOAc fraction did not exhibit any effective inhibitory action. The detected α-amylase inhibitory activity was comparable to that of acarbose (IC_{50}=14.88 μg/mL). It should be mentioned that acarbose has been used for management of post-prandial hyperglycemia but it was reported that this agent was associated with several health side effects[10].

The potent α-amylase inhibitory activity of the hexane and the methanol extracts depended on the amount of total phenolics and flavonoids in each extract. In fact, many phenolic compounds and specially flavonoids have been reported as potential antidiabetic agents because they exert a good inhibitory action against α-amylase and could have potential prevention in diabetes mellitus as part of a dietary strategy[29]. Furthermore, the terpenes such as α-pinene which exist in *J. phoenicea* essential oil might inhibited key enzymes related to Type 2 diabetes principally α-amylase. It was reported that administration of terpenes to diabetic exerts blood glucose lowering effect and high antioxidant activity in alloxan-induced diabetic rat[30].

The inhibitory effect of *J. phoenicea* extracts against pancreatic lipase was compared to that of orlistat (IC_{50} of 0.85 μg/mL), a lipase inhibitor used as positive control. Except EtOAc extract, all the tested samples exhibited a powerful inhibitory of the enzyme activity in the following croissant order: hexane extract<essential oil extract<methanol extract. Actually, obesity is caused by excess caloric intake[31], and this can be remedied by inhibiting pancreatic lipase activity and by inhibiting or delaying lipid absorption[32]. The inhibitive capacities of the different extracts of *J. phoenicea* against the lipase activity might be perfectly coincident with their total phenolics compounds. In fact, various natural products containing polyphenols have been reported to have anti-obesity and anti-diabetic effects by inhibiting lipase activity and α-glucosidase activity[33,34]. McDougall and co-workers have established that the inhibitory lipase activity might be derived from the phenolic compounds found in some medicinal plants such as gallic acid, catechin, epicatechin, ellagic acid, myricetin, quercetin, kaempferol, resveratrol, and anthocyanin[35].

Our results demonstrated for the first time the potent inhibitory effect of various extracts of *J. phoenicea* leaves as well as its essential oil against key enzymes related to diabetes and obesity. Furthermore, this investigation showed a notable relationship between total phenolic contents and α-amylase, pancreatic lipase inhibitory activities of the *J. phoenicea* extracts as well as their antioxidant activity. Further studies are needed for the isolation and identification of individual natural compounds and the assessment of their antioxidant, anti-diabetic and anti-obesity activities.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

This research was supported by the Ministry of High Education and Scientific Research, Tunisia.

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