

Evaluation of In-vitro Antidiabetic and Hypolipidaemic Activities of Extracts *Citrus Lemon* Fruit

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Abstract: Citrus genus is the most important fruit tree crop in the world and lemon is the third most important Citrus species. The evaluation of the potential inhibitory activity on α -amylase and pancreatic lipase by *Citrus lemon* fruits of Algerian origin aiming for new applications of the fruits in nutrition and health was carried out. Lemon fruit EtOAc (ethyl acetate) extract showed higher contents of phytochemicals such as total phenolics and flavonoids. Furthermore, lemon fruit showed higher potential for inhibitory effects on α -amylase and pancreatic lipase. It could be concluded that lemon fruits are of great value for nutrition and treatment of diet-related diseases such as obesity and diabetes.

Key words: Diabetes, flavonoids, obesity, polyphenols.

1. Introduction

Diabetes mellitus is a clinical syndrome characterized by inappropriate persistently elevated blood glucose concentration caused by deficiency of insulin at the cellular level, leading to acute or long term complications [1]. Globally, diabetes mellitus presents enormous and increasingly important public health issues. The prevalence of diabetes continues to rise and at the present, more than 220 million people worldwide have diabetes and the number of diabetes deaths will double in 2030 [2]. Currently, available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides and α -glycosidase inhibitors. These agents, however, have restricted usage due to several undesirable side effects and fail to significantly after the course of diabetic complications [3]. Moreover, diabetes is associated with increased risk of thrombotic, atherosclerotic and cardiovascular disease [4]. It is well

established that patients with diabetes type 2 frequently have abnormal serum lipid profile. The dyslipidemia is characterized by low levels of HDL (High-Density Lipoprotein)-cholesterol, and high levels of LDL (Low-Density Lipoprotein)-cholesterol and TGS (Triglycerides) [5, 6]. Studies have suggested that excessive intake of calories are related to chronic diseases, including type 2 diabetes mellitus, CVD (Cardiovascular Disease) and obesity. These are all linked to oxidative stress, causing an imbalance of pro oxidants and antioxidants in cellular systems, which impairs normal biological functions [7, 8].

Inhibition of α -amylase, enzyme that plays a role in digestion of starch and glycogen, is considered a strategy for the treatment of disorders in carbohydrate uptake, such as diabetes and obesity. A wide array of numerous chemical compounds has demonstrated activity consistent with their possible use in the treatment of diabetes [9-12]. Different plants have been reported to show α -amylase inhibitory activity and so may be relevant to the treatment of diabetes [13, 14]. The potential role of the medicinal plants as inhibitors

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of α -amylase has been frequently assigned to phenolic compounds which possess various degrees of antioxidant or free radical scavenging properties as well as medicinal properties and have long been used as drugs [15-18]. Furthermore, flavonoids, abundant class of natural phenolic compounds showed that the potency of α -amylase inhibition is correlated with the number of hydroxyl groups on the B ring of the flavonoid skeleton [19].

Citrus lemon is an important medicinal plant of the family Rutaceae and it is a very popular fruit all over the world due to the taste, aromatic flavor and healing properties [20, 21]. In Algeria, *C. lemon* is among the most popular fruits grown and it is an important source of photochemical such as vitamin c, phenolic compounds, many of which have potent antioxidant activities which are often exploited in food products and in various medicinal treatments [22, 23]. A significant fraction of flavonoids was found in *C. lemon* which includes hesperidin, quercitrin, eriocitrin, didymin and naringin [24]. The role of naringin has been reported for its antidiabetic effects [25]. *C. lemon* flavonoids have also shown having a broad spectrum of biological activity including antimicrobial, anticancer and antiviral activities [26-30].

The present investigation was undertaken to evaluate the in vitro inhibition activities antidiabetic and hypolipidemic using α -amylase and pancreatic lipase of EtOAc extract fruit of *C. lemon*.

2. Material and Methods

2.1 Material

Fresh fruit used in this study were obtained on the local market at city Bejaia, Algeria, 2014. Fresh fruits were washed in tap water running in the laboratory, rinsed with sterile distilled water. The pressed juice was put into a vessel separately, and then the fruits were cut with a sterile knife, dried in dry and powdered.

2.2 Extraction

Five grams of fruit *C. lemon* powder were extracted

for 12 h with 200 mL of 70% hydro-alcoholic solvent (methanol/water) at room temperature. The extract was filtered, and then the residue was extracted for the second time with 100 mL of the same hydro-alcoholic solvent for 12 h at room temperature. After removal of methanol under reduced pressure in a rotary evaporator at 40 °C, the remaining aqueous solution of the extraction was defatted twice with petroleum ether to remove lipids. Then, the aqueous fraction was extracted with ethyl acetate. The organic fractions evaporated to dryness using a rotary evaporator. The dried residue was dissolved in of methanol and kept at 4 °C.

2.3 Determination of Total Phenolic Compound

The amount of total phenolic in the sample was determined with the Folin-Ciocalteu reagent using the method of Singleton and Ross (1965) [31]. 100 μ L of EtOAc lemon extract was added to 500 μ L of the aqueous solution of Folin-Ciocalteu reagent at 10%. After 2 min incubation at room temperature, 2 mL of 2% (w/v) sodium carbonate in water were added. Blank was prepared by replacing the reagent by water to correct for interfering compound. After 30 min of incubation in the dark at room temperature, the absorbance was measured at 760 nm using the Shimadzu 1601 visible spectrophotometer. The gallic acid was used as a standard and the results were expressed as milligram of GAE (Gallic Acid Equivalents) per gram dry extract. The entire assay was carried out at least in triplicate.

2.4 Determination of Total Flavonoids

Total flavonoids content in the citrus fruit extract was determined spectrophotometrically using a method based on the formation of a flavonoid-aluminium complex with an absorbance maximum at 430 nm [32]. The examined extract (1 mL) was mixed with 2% $\text{AlCl}_3 \times 6\text{H}_2\text{O}$ (0.5 mL). After incubation at room temperature for 30 min, the absorbance of the reaction mixtures was measured. The blank sample was a 1:1

mixture of the examined extract and distilled water. Flavonoids content was expressed in μg quercetin equivalent per gram dried extract by using a standard curve of quercetin (concentration range 0.5-6.0 $\mu\text{g}/\text{mL}$). All measurements were replicated for three times.

2.5 In vitro α -amylase Inhibitory Activity

This study was performed by a modified starch iodine protocol [33]. In short, 80 μL of EtOAc lemon extract or standard (acarbose) of different concentration was taken in pre-labeled test tubes. A volume of 20 μL of α -amylase was added to each test tube and incubated for 10 min at 37 °C. After the incubation 200 μL of 1% starch solution was added to each test tube and the mixture was re-incubated for 1 h at 37 °C. Then 200 μL of 1% iodine solution was added to each test tube and after that, 10 mL distilled water was added. Absorbance of the mixture was taken at 565 nm. The experiments were repeated thrice and the percentage inhibition was calculated by the expression:

$$\% \text{ inhibition} = \frac{\text{Absorbance Control} - \text{Absorbance Test}}{\text{Absorbance Control}} \times 100 \quad (1)$$

2.6 Lipase Inhibitory Activity

Inhibition of lipase by the EtOAc lemon extract was determined using a modified assay [34]. Briefly, a suspension containing 1% (v/v) of triolein, and 1% (v/v) Tween 40 in 0.1 M phosphate buffer (pH 8) was prepared and emulsified. Assays were then initiated by adding 800 μL of the triolein emulsion to 200 μL of pancreatic lipase and 200 μL of extract (or 0.1 M Phosphate buffer, pH 8). The contents were mixed and the absorbance measured immediately at 450 nm and designated as T_0 . The test tubes were incubated at 37 °C for 30 min and at the end of the incubation; the absorbance at 450 nm was recorded and designated as T_{30} .

The variation in absorbance = $[A_{450}(T_0) - A_{450}(T_{30})]$ was calculated for both control and the test and the % inhibition was calculated using the formula:

$$\% \text{ inhibition} = \frac{(\Delta A_{450} \text{ control} - \Delta A_{450} \text{ Extract})}{(\Delta A_{450} \text{ control})} \times 100 \quad (2)$$

3. Results and Discussions

3.1 Polyphenols and Flavonoids Content

Analysis of Folin-Ciocalteu index is one of the oldest methods developed to determine the content of total phenols. The average values of total phenols ranged from 63.9 ± 0.16 mg GAE/g and 12.3 ± 0.30 mg of flavonoids QE/g. These results agree with other studies which have shown that lemon was found to contain the highest total phenolic and flavonoids contents [35-39]. Some authors have even reported that the total polyphenol content in the peeled lemons and their peels are significantly higher than those in oranges and grapefruits, peeled and peels, respectively [38]. In addition, total phenolics and total flavonoid content were still significantly higher in the skin than those in the pulp for all citrus fruits, in accordance with previous studies [40, 41]. Therefore, citrus peels have been widely studied because they contain many biologically active compounds, including natural antioxidants such as phenolic acids and flavonoids [42, 43]. Flavonoids are the most abundant phenolic compounds present in Citrus fruits. The content of flavonoids in peels is 10-time greater than the content of the juice. Among them, flavanone glycosides predominate, together with other low amount flavonoids such as methoxylated flavones, flavones glycosides and anthocyanins [44, 45]. Then, the consumption of fruit and citrus juice has been widely studied for its possible role in promoting health and preventing human disease. These beneficial effects are mainly due to flavanones, polyphenols typical citrus species [46-48].

3.2 Inhibition of α -Amylase Activity

Extract EtOAc showed a potent inhibition of α -amylase enzyme. In fact, the IC50 values of Citrus

lemon fruit extract and standard acarbose against α -amylase were 103.46 $\mu\text{g/mL}$ and 134.17 $\mu\text{g/mL}$, respectively. Lemon extract significantly inhibited α -amylase activity in a dose dependent manner like acarbose. The result of in vitro α -amylase inhibitory activity of lemon fruits extract was shown in Table 1.

The potent α -amylase inhibitory activity of the EtOAc lemon extract depended on amount of total phenolic and flavonoids in extract. Recent studies have shown that phenolic phytochemicals exert anti-diabetic effect through inhibition of carbohydrate-hydrolyzing enzymes, such as alpha-amylase. 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 [49]. These results were confirmed by others studies which showed that all of the tested Citrus flavonoids significantly inhibited amylase-catalyzed starch digestion [50]. Natural alpha-amylase inhibitors offer an attractive approach to the management of postprandial hyperglycemia by

decreasing glucose release from starch [51]. Moreover, several findings suggest that phenolic synergies may play a role in mediating amylase inhibition and therefore have the potential to contribute to the management of type 2 diabetes [52].

3.3 Pancreatic Lipase in vitro Assay

The EtOAc lemon extract as well as Orlistat, a pancreatic lipase inhibitor used as an anti-obesity agent exhibited a strong inhibition pancreatic lipase in vitro activity. They inhibited the enzyme activity with IC_{50} corresponding to 110.07 and 98.40 $\mu\text{g/mL}$, respectively. The inhibition activity of lemon extract and Orlistat against pancreatic lipase was significantly similar and shown in dose-dependent manner. The inhibitory activity towards pancreatic lipase was reported in Table 2.

Obesity is found to be strong risk factor for type 2 diabetes. Dietary lipids represent the major source of unwanted calories; therefore, lipid metabolism is a vital and subtle balance that maintains energy homeostasis. A vast range of health problems co-exist with a weight problem and dysfunction of lipid homeostasis.

Table 1 In vitro α -amylase inhibitory activity for *C. lemon* EtOAc fruit extract and acarbose.

Sample	Concent ($\mu\text{g/mL}$)	%Inhibition	IC_{50} $\mu\text{g/mL}$
Acarbose	50	31.53 \pm 0.14	103.46
	150	72.49 \pm 0.09	
	300	82.92 \pm 0.12	
EtOAc	50	38.02 \pm 0.1	134.17
	150	55.9 \pm 0.11	
	300	63.14 \pm 0.1	

Values are the mean of triplicate experiments and represented as mean \pm SEM (n = 3).

Table 2 In vitro lipase inhibitory activity of EtOAc fruit lemon extract.

Sample	Concent ($\mu\text{g/mL}$)	%inhibition	IC_{50} $\mu\text{g/mL}$
Orlistat	25	21.09 \pm 0.1	98.40
	100	32.15 \pm 0.05	
	125	63.52 \pm 0.02	
	250	86.33 \pm 0.9	
	25	21.50 \pm 0.1	
EtOAc	100	45.63 \pm 0.01	110.07
	125	56.78 \pm 0.15	
	250	74.52 \pm 0.09	

Values are the mean of triplicate experiments and represented as mean \pm SEM (n = 3).

Pancreatic lipase is the key enzyme for dietary fat digestion [53], and inhibition of the enzyme could be an effective way to alter fat absorption. In fact, Orlistat, an appetite suppressor, is the main anti-obesity medications currently approved [54]. However, because Orlistat can result in undesirable side effects, such as fecal incontinence, flatulence and steatorrhea, its use may be limited [55]. It may be worthwhile to search the natural substances that show potent inhibitory activity. So far, many natural products (plant extracts and isolated compounds) have been reported for their pancreatic lipase inhibition property. It has been shown that Citrus fruits inhibited pancreatic lipase activity dose dependently and are of great value for nutrition and treatment of diet-related diseases such as obesity and diabetes [56]. The lipase inhibitory effect observed on Citrus *sp.* such as lemon could be due to the acidic plant extracts which having pH values below 4.0 [57].

4. Conclusion

It can be concluded that fruit of *Citrus lemon* can be promising an antidiabetic and hypolipidaemic. This verifies the traditional use of this plant as hypoglycaemic agents. Ideally, such research will lead to a more effective and safer pharmacological treatment of diabetes and obesity.

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