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In Vitro α-Amylase Inhibition of Essential Oil Obtained from Laurel (*Laurus nobilis* L.) Leaves

Serap Şahin Başak* **and Ferda Candan**

Cumhuriyet University, Faculty of Science and Literature, Department of Chemistry, Sivas, Turkey

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Abstract

The present study was designed to determine in vitro antidiabetic properties of the essential oil obtained from the leaves of Laurel (*Laurus nobilis L.*) which is traditionally used for the treatment of diabetes. With this objective we study the α-amylase inhibition property of *Laurus nobilis* L. essential oil which is obtained from the leaves using a Clevenger distillation apparatus. We find that the essential oil of laurus inhibits α-amylase and the type of inhibition is competitive inhibition.

INTRODUCTION

Diabetes (Diabetes mellitus (DM)) is a disease which arises from total or partial insulin deficiency, and which is also characterized by high blood sugar (hyperglycemia). Besides insulin deficiency, the resistance that rises against insulin also contributes to the development of diabetes and effects carbohydrate, lipid and protein metabolisms [1] Uncontrolled high blood sugar increases the risk of paralysis, gangrene and coronary diseases as a result of long term complications such as nephropathia which results in kidney failure, nervous system disease (neuropathy), retinosis which may result in blindness, and foot ulcer [2].

* Correspondence to: Serap ŞAHİN BAŞAK

Department of Chemistry, Faculty of Science and Literature, Cumhuriyet University, Sivas 58140 Turkey

Tel: +90 346 219 1010 / 2143 Fax: +90346 219 1186
E-mail: wserap@yahoo.com

Diabetes is investigated under two types; Type I and Type II. Insulin dependant diabetes is classified as 'Type I'. Its primary property is that no insulin or only very little insulin is secreted depending on the failure degree of the beta cells of the islets of Langerhans in the pancreas, [3].

The other type of diabetes is not dependant on insulin and is classified as 'Type II'. In this type, contrary to Type I, insulin deficiency is not in question. Insulin is secreted in normal, and sometimes even in high amounts. Nevertheless, in this case the insulin production of the pancreas is not sufficient, and the insulin is left inefficient. Thus, the problem can be defined as insufficiency or inefficiency of insulin [4].

The current diabetes therapies include some modifications in life styles, such as diet, exercise,

and the use of diverse pharmacological agents. The medicines used for the treatment of diabetes have negative side effects because of the fact that they are synthetic and that with high doses they contain the risk to effect liver function disorders. For this reason, in antidiabetic therapy, the development of natural agents which will be effective against hyperglycemia is focused on [5,6].

The use of plants to treatment of diabetes, according to the information conveyed on Ebers papyrus, starts from B.C.1500 [7]. Various plants are used all around the world to treatment of diabetes [8], especially in traditional Chinese and Indian medicine, herbal antidiabetic agents extracted from plants are being used for a long time [9,10]. According to ethnobiological information, more than 800 plants are being used in traditional diabetes treatments [11].

Laurus nobilis L. is a plant which belongs to the *Lauraceae* family. The kind we have used in our study is the leaves of *Laurus nobilis L.* (Lauraceae). This kind is 3-10 meters in height, and is a tree with yellow flowers, which does not shed its leaves in winter; its leaves are 5-10 cm long and 2-5 cm wide, with hard and short stems, green in color; and its fruits are in the form of small olives. While being widespread in Europe, it is found in our country in the Aegean, Mediterranean and the Black Sea coastal regions [12].

It is traditionally used as an anticeptic, antirheumatismal, diuretic, ear and stomach pain killer cure; its dry fruits are used as sweetener in food, dry leaves are consumed as tea, and essential oil is used in the production of soap and also as an aroma in food and cosmetics industries [12]. The antimicrobial, antifungal, insect repellent, analgesic, anti-inflamatory, antitumoural, acetylcholine esterase inhibition activity and anticonvulsive properties of the essential oil of the plant has been reported [13-17].

One of the therapeutic approaches to lower postprandial blood sugar is delaying the absorption of glucose by inhibiting carbohydrate hydrolysis enzymes such as α-amylase and α-glucosidase in the digestive system organs [18]. α-amylases (E.C 3.2.1.1) are the key enzymes working for the digestion of carbohydrates.

α-amylases (α-1,4-glucan-4-glucanohydrolase) which are classified as enzymes that hydrolyse glycoside bonds of glycogen, amylopectin and amylose α-D-(1,4), are commonly present in many autotroph and hetotroph organisms, and have an important role in carbohydrtae metabolisms. α-amylase (α-1→6) is not effective in glycoside bond. It is reported that since the inhibition of α-amylase results in carbohydrate tolerance, feelings of satiety, weight loss and long term gastric emptying. Thus α-amylase inhibitors may be effective in the treatment of obesity and Type II diabetes [19,20].

Within the context of this research, the traditionally widespread used in our country of laurel (*Laurus nobilis* L.) essential oil in order to lower the high blood sugar observed in diabetes, effects on α-amylase and also its inhibitions kinetics was studied. In this study, an α-amylase inhibition in vitro model is used to screen the essential oil of *Laurus nobilis* L. to evaluate its potential hypoglycemic effects.

MATERIALS AND METHODS

Chemicals

Patato starch, disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium chloride, porcine pancreatic α-amylase (30.000 U), n-hexane, sodium hydroxyl, sodium potassium tartarate. tetrahydrate and glucose were purchased from Merck (Darmstad, Germany). 3,5-dinitrosalicylic

acid was purchased from Sigma (Steinheim, Germany).

Plant Material

Laurus nobilis L. leaves, collected in June 2006 from İstanbul in Turkey, was identified in the Biology Department of Cumhuriyet University and voucher specimen is deposited in Chemistry Department of Cumhuriyet University Biochemistry Research Laboratory II.

Extraction of the Essential Oil

The leaves of *Laurus nobilis* L. shade-dried and submitted for 3 h to water distillation using a Clevenger-type apparatus (yield 0.856% v/w). The obtained essential oil was stored at +4°C until tested and analyzed.

α-Amylase İnhibitory Assays

The bioassay method was adopted and modified from Sigma-Aldrich [21]. A starch solution (0.5% w/v) was obtained by stirring 0.125 g of potato starch in 25 ml of 20 mM sodium phosphate buffer with 6.7 mM sodium chloride, pH 6.9 at 65°C for 15 min. The enzyme solution was prepared by mixing 0.0253 g of (α**-**amylase in 100 ml of cold distilled water. *Laurus Nobilis* L. essential oil dissolved in n-hexane. The colorimetric reagent was prepared mixing a sodium potassium tartarate solution (12.0 g of sodium potassium tartarate, tetrahydrate in 8.0 ml of 2 m NaOH) and 96 mM 3,5-dinitrosalicylic acid solution. Both control and plant essential oil were added with starch solution and left to react with α**-**amylase solution under an alkaline condition at 25°C. The reaction was measured over 3 min.

The generation of maltose was quantified by the reduction of 3,5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid. This reaction (corresponding to colour change from orange-yellow to red) is detectable at 540 nm. In the presence of an (α-amylase inhibitors less maltose would be produced and the absorbance value would be decreased.

Kinetic studies of *Laurus nobilis* L using Michaelis-Menten and Lineweaver-Burk equations.

A calibration curve was drawn using the 'glucose assay by 3-5,dinitrosalicyclic acid (DNS) colorimetric method [22] with minor modifications. To 3 mL of graded concentrations of glucose solution (0.625-10 g/L) was added 3 mL of DNS acid reagent solution, 1% DNS (DNS 10 g, sodium disulphite 0.5 g and sodium hydroxide 10 g in 1 L distilled water). The test tubes were then covered with a paraffin film. The mixture was heated at 90°C for 5-15 min until a red-brown colour developed. 1 mL of a 40% potassium sodium tartarate solution was then added. The test tubes were cooled under tap water and the absorbance was measured at 575 nm.

Control Experiments

All reagents were pre-incubated for 15 min at 37°C in a water bath. To 0.5 mL of graded concentrations of starch solution [(1-50 g/L) in 0.2 M sodium phosphate buffer at pH 7.0] and 0.25 mL of the buffer was added 0.25 mL of α-amylase enzyme solution (30 µg/mL in 0.2 M sodium phosphate buffer, pH 7.0, and containing 6 mM NaCl). At the end of 3 min, the reaction was stopped by the addition of 2 mL DNS reagent and heating in a boiling water bath at 90°C for 10 min. 1 mL of a 40% potassium sodium tartarate solution was then added. The test tubes were cooled under tap water and the absorbance was measured at 575 nm.

Incorporation of the Inhibitor

0.25 mL of essential oil of *Laurus nobilis L*. (in hexane) was incubated with 0.25 mL of α-amylase enzyme solution for 15 min at 37°C. The above procedure was then repeated.

Michaelis-Menten and Lineweaver-Burk Equations.

Interms of enzyme kinetics, the Michaelis-Menten constant (Km) and maximum velocity (Vmax) in the

presence and absence of essential oil of *Laurus nobilis* L. were determined from the Michaelis-Menten and Lineweaver-Burk equations.

Statistics

Data were expressed as means±S.D. of three determinations. Statistical analyses were performed using Sigma Plot graphs / istatistical programme and Student's t-test analysis. Differences were considered significant at $p < 0.01$. The inhibitory concentration calculated from the Sigma Plot dose-response curve (statistical programme) obtained by plotting the percentage of inhibition versus the concentrations.

RESULT AND DISCUSSION

In the control of diabetes mellitus drugs have helpfull for decrease of postprantial high blood sugar level by inhibited the hydrolysis of carbohydrates enzymes [18]. In the present study, essential oil of *Laurus nobilis* L. was found to possess significant inhibitory effects *(p* < 0.01) on starch break down *in vitro* as shown in Table 1. The α-amylase inhibitions percent of the laurel essential oil was determined 42.12 ± 2.36. Because laurel essential oil does not have the activity to inhibit α-amylase by %50, IC_{50} (concentration of essential oil for 50 percent inhibition of α-amylase) could not be observed.

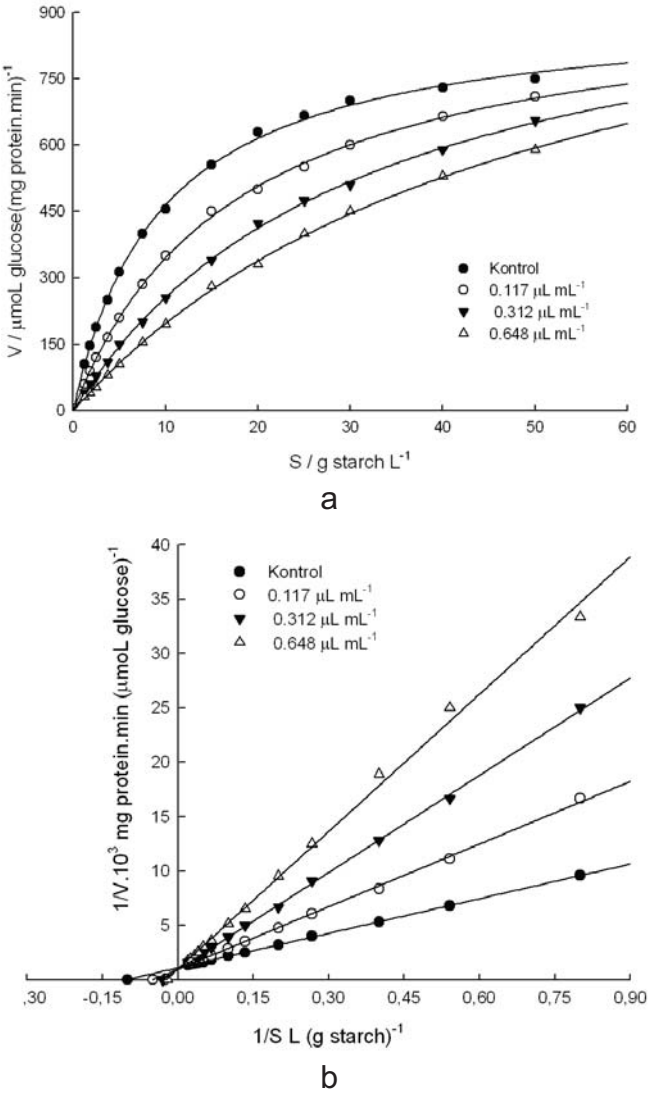

Figure 1. The a) Michealis-Menten and b) Lineweaver-Burk plot in the absence and presence of essential oil of *Laurus nobilis* L. (○, ●, ►, Δ are presented different concentration of laurel essential oil)

Table 1. Inhibitory activity of laurel essential oil against α-amylase

Sample	Concentration (µL mL^{-1})	% Inhibition[a]
Essential oil	0.093	16.00 ± 0.83
	0.186	26.00 ± 1.12
	0.372	36.80 ± 0.65
	0.465	40.00 ± 1.25
	0.559	41.00 ± 0.32
	0.745	42.12 ± 2.36

[a] All determinations were carried out in triplicate and averaged. The amylase inhibitory activity (%) was defined as the percent decrease in the maltose production rate over the control.

Table 2. V_{max} ve K_m value of only starch and starch with different concentration of laurel essential oil

Essential oil	Concentration (µL mL^{-1})	Vm[a] µmoLglucose (mg protein.min)$^{-1}$	K_m[a] (g starch L^{-1})
Laurel	Control	975.12 ± 1.73	9.565 ± 0.025
	0.117	975.12 ± 3.05	19.982 ± 0.051
	0.312	975.12 ± 1.53	26.323 ± 0.125
	0.648	975.12 ± 1.00	38.216 ± 0.115

[a] Values are expressed as mean ±SD

The catalytic studies using the Michaelis-Menten and Lineweaver-Burk equations were performed (Figure. 1).

It was observed that while the maximum velocity (V_{max}, y-intercept remained) in laurel essential oil unchanged and the Michaelis-Menten constant the (K_m , slope of the trend lines) value increased (Table 2). So the type of inhitibion is competitive inhibition. This result showed that, the essential oil of *Laurus nobilis* L. was acting as a competitive inhibitor of the enzyme. The competitive inhibitors are competing with substrate to bond with the active region of the free enzyme, and cause inhibition by forming an enzyme-inhibiting complex. For this reason, the inhibition can be prevented by increasing the concentration of substrate. When the Km values of laurel essential oil graphed, concentrations of laurel essential oil which demonstrates competitive inhibition by Smallest Squares Method, concentration of laurel essential oil as (Ki) value was determined as 0.34 + 0.02 µL mL^{-1}.

The results convey that the essential oil obtained from laurel leaves inhibit α-amylase In conclusion, since the essential oil obtained from the leaves of laurel have inhibited α-amylase, laurel essential oil could be effective in the treatment of diabetes. But, the results needed to be tested by in *vivo* experiments.

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